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CONTENTS

PAGE

BIOLOGICAL FOOD TESTS. IV. THE PROTEIN AND THE VITAMIN—A CONTENT OF THE ENGLISH WALNUT. *Helen L. Mignon*..... 215
ON THE SOURCES OF HEAT IN MUSCLE CONTRACTION. *Charles D. Snyder*..... 232
THE AVERAGE EPINEPHRIN OUTPUT IN CATS AND DOGS. *G. N. Stewart and J. M. Rogoff*. 235
ON THE FUNCTION OF THE CEREBRAL VENTRICLES. I. DEMONSTRATION OF SMALL CHANGES IN THE CAPACITY OF THE LATERAL VENTRICLES OF THE BRAIN. *K. J. Austmann and V. H. K. Moorhouse*..... 267
THE RÔLE OF ANOXEMIA IN THE CAUSATION OF TETANY DURING HYPERPNEA. *Samuel B. Grant*..... 274
THE RELATIONS BETWEEN LESIONS OF THE BRAIN STEM, WATER ELIMINATION AND BODY TEMPERATURE. (PRELIMINARY REPORT.) *Fred T. Rogers*..... 284
THE GILL MOVEMENTS IN ONE OF THE PERENNIBRANCHIATE URODELA (NECTURUS MACULATUS) AND THEIR RELATION TO THE CENTRAL NERVOUS SYSTEM. *G. N. Stewart*. 288
THE QUANTITATIVE DETERMINATION OF TRYPSIN AND LIPASE IN GASTRIC CONTENTS. *C. W. Lueders and Olaf Bergeim*..... 297
THE EFFECT OF ASPHYXIA UPON THE ADRENAL OUTPUT AS DEMONSTRATED BY VARIATIONS IN THE TEMPERATURE OF THE BRAIN. *George W. Crile, Amy F. Rowland and S. W. Wallace*..... 304
STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XIII. ASPHYXIAL DEATH OF EMBRYOS IN EGGS ABNORMALLY RETAINED IN THE OVIDUCT. *Oscar Riddle*..... 309
STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XIV. SUPRARENAL HYPERTROPHY COINCIDENT WITH OVULATION. *Oscar Riddle*..... 322
STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. XV. INCREASED BLOOD SUGAR COINCIDENT WITH OVULATION IN VARIOUS KINDS OF PIGEONS. *Oscar Riddle and Hannah Elizabeth Honeywell*..... 340
THE MINERAL METABOLISM OF THE LACTATING AND DRY GOAT. *Chas. H. Hunt, A. R. Winter, J. A. Schultz and R. C. Miller*..... 349
THE EFFECT OF DIURESIS ON EXCRETION OF URIC ACID. *J. Lucien Morris and H. Maynard Rees*..... 363
THE PRODUCTION OF HYPOGLYCEMIA IN EXPERIMENTAL DERANGEMENTS OF THE LIVER. *Meyer Bodansky*..... 375
STUDIES IN PERISTALTIC FATIGUE. *Velyien E. Henderson*..... 380
REFLEX CONTRACTIONS OF AN ALL-OR-NONE CHARACTER IN THE SPINAL CAT. *Eugene L. Porter and Victor W. Hart*..... 391
FURTHER OBSERVATIONS ON THE ANTIGENIC EFFECTS OF SEMEN. MECHANISM OF STERILIZATION OF FEMALE RAT FROM INJECTIONS OF SPERMATOZOA. *J. L. McCartney*..... 404
ADRENAL DISCHARGE IN THE DOG. *Jackson Searles*..... 408
FURTHER STUDIES OF THE REACTION OF THE SMALL INTESTINE TO SODIUM BICARBONATE. *C. E. King and Jas. G. Church*..... 414
THE MOTOR REACTION OF THE MUSCULARIS MUCOSAE TO SOME DRUGS. *C. E. King and Jas. G. Church*..... 428
THE EFFECT OF INSULIN ON THE CENTRAL NERVOUS SYSTEM AND ITS RELATION TO THE PITUITARY BODY. *J. M. D. Olmsted and H. D. Logan*..... 437

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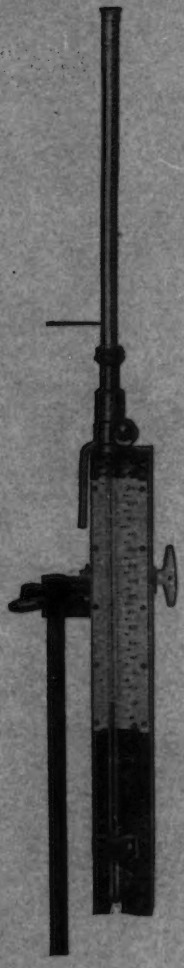

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BIOLOGICAL FOOD TESTS

IV. The Protein and the Vitamin A Content of the English Walnut

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THE PROTEINS OF THE ENGLISH WALNUT. Considerable attention has been given to the investigation of nut proteins. Many such proteins have been studied chemically, and a few have been studied biologically as well. Only a few experiments have dealt in either of these ways with the proteins of the English walnut. Osborne and Harris (1) reported a study of walnut globulins in connection with studies on other nuts. The globulins of the various varieties of walnuts were found to be similar, and the name *juglansin* was bestowed upon the globulins of the Juglans species. These proteins possessed a high content of basic amino nitrogen and gave a strongly positive test for tryptophane. Cajori (2) reported that mixed walnut proteins fed as the sole source of dietary protein were adequate for normal development in rats.

The present investigation was undertaken to determine the adequacy of walnut globulin as a sole source of dietary protein for white mice, as well as to determine the adequacy of the mixed walnut proteins at different levels of intake for the normal development of rats and mice.

EXPERIMENTAL METHODS. *1. Mixed walnut proteins: Preparation of the mixed proteins.* The soft-shell English walnut, sold under a given trade name, was used throughout the experiment. The nuts were shelled and blanched by hand. This was a slow and tedious process, but preliminary experiments indicated that the tannin present in the skin was a limiting factor in the extraction of the globulin and, if fed in large quantities, was a limiting factor in the normal growth of the animals. The lye method, which Cajori (3) was able to use for the

pecan nut, was not found satisfactory for the walnut either from the standpoint of time or effectiveness. The hand process, using boiling water, was found highly satisfactory.

The blanched nuts were ground in a meat grinder, transferred to a muslin bag, and as much oil as possible was pressed out by hand, using the ordinary type of tincture press. The resulting cake was broken up, placed in double cloth bags, and extracted with U. S. P. ether in a large fat extractor of the Soxhlet type (4). The extraction was continued until all but traces of fatty matter were removed. The ether was allowed to evaporate from the residue meal, leaving a cream-colored dry powder, having nitrogen content 7.2 per cent (protein 45 per cent) as determined by the Kjeldahl method.

Feeding experiments with walnut meal as sole source of protein. Two rats and three mice were fed this meal as the sole source of protein in an otherwise adequate diet, using an 18 per cent level of protein intake. The animals were taken at weaning, and the experiment continued 125 days. The diet was made up as follows:

Fat-free walnut meal (7.2 per cent N)	40
Salts (Osborne and Mendel)	4
Baked starch	36
Crisco	10
Butterfat	10
Dried brewery yeast,	
Rats	300 mgm. daily
Mice	100 mgm. daily

It was found unnecessary to use agar with the whole meal present in such large amounts. All the animals ate the food heartily. They grew normally, were very active, and had fine sleek coats of fur.

Three rats and two mice were fed a diet which gave a 12 per cent level of intake of the mixed proteins.

Fat-free walnut meal (7.2 per cent N)	27
Salts (Osborne and Mende[1])	4
Baked starch	48
Agar	1
Crisco	10
Butterfat	10
Dried brewery yeast,	
Rats	300 mgm. daily
Mice	100 mgm. daily

All the animals grew normally and were comparable with the controls and with those animals fed upon a higher level of protein intake.

Four rats and two mice were fed a diet which gave a 9 per cent level of intake of the mixed proteins.

Fat-free walnut meal (7.2 per cent N)	21
Salts (Osborne and Mendel)	4
Baked starch	53
Agar	2
Crisco	10
Butterfat	10
Dried brewery yeast,	
Rats	300 mgm. daily
Mice	160 mgm. daily

The animals grew rather well, but they showed greater individual variations and more often approached the lower limits of normal growth than did those fed the higher levels of protein. The author feels that the margin was very close, and that, for entire safety, most rats and mice would require a higher level (12 per cent) of intake of mixed walnut proteins.

2. Walnut globulin. The methods used for the isolation and purification of the globulin were similar to those used by Osborne and Harris (1), Johns and co-workers (5), Cajori (3) and others. Preliminary experiments were made to determine the relative amount and kind of proteins extracted by different strengths of salt solution (U. S. P. sodium chloride).

Salt solution extracts. Ten-gram lots of fat-free walnut meal were extracted with ten times their weight of 8, 10 and 12 per cent salt solutions respectively for 24 hours at approximately 4 to 10°C. The extracts were filtered through three thicknesses of clean muslin, then through paper pulp on a Buchner funnel. Total nitrogen was determined on an aliquot part of the filtrate by the Kjeldahl method, and the amount of protein extracted was calculated. A 10 per cent salt solution was found most efficient and was used for globulin extraction throughout the experiments.

Coagulation temperature of salt solution extracts. Twenty-five cubic centimeters of a 10 per cent salt solution extract of fat-free meal were faintly acidified with 2 per cent acetic acid. The solution was carefully heated in a water jacket at the rate of 1° rise in temperature in 2 minutes.

At 55° there was a slight clouding reaching a maximum at 65°. The solution was held at 60 to 65° for 1 hour, cooled and filtered. This gave a small amount of coagulum which was judged to be albumin.

The filtrate was heated again as before. A slight cloud formed above 75°. The solution was held at 80 to 85° for 1 hour, cooled and filtered. This gave a small amount of coagulum only, and unless a very concentrated solution was used, practically no coagulum could be detected.

The filtrate, heated again, clouded above 90°, a heavy coagulation taking place at 95 to 100°. The solution was held at 100° for 1 hour,

cooled and filtered. This gave the largest protein fraction which was judged to be the chief globulin of the walnut. The filtrate remaining contained but slight traces of nitrogen.

Fractional precipitation with ammonium sulfate. Ten cubic centimeters of a 10 per cent salt solution of walnut globulin were treated with calculated amounts of finely powdered ammonium sulfate so as to increase the saturation of the solution gradually. The solution was shaken until the salt completely dissolved and was examined for protein precipitate before each addition.

At two-tenths saturation a slight cloud appeared increasing at three-tenths saturation. The precipitate was allowed to stand 10 to 15 minutes and was then filtered off. At four-tenths saturation more cloud appeared increasing to a very heavy precipitation at five-tenths saturation. This was allowed to stand as before and then filtered off. No further clouding occurred until eight-tenths saturation, when a slight cloud appeared and persisted to complete saturation.

Conclusions drawn from preliminary experiments. The chief globulin of the walnut coagulates at 95 to 100° in a faintly acidified solution and is precipitated from salt solution by five-tenths saturation with ammonium sulfate. Some albumin is present which coagulates between 55° and 65° and is precipitated from salt solution by complete saturation with ammonium sulfate. Some indication was found of a slight amount of another protein fraction coagulating between 75° and 85°, but no successful precipitation with ammonium sulfate was obtained which would allow of its isolation.

The preparation of the globulin. Four hundred gram lots of fat-free meal were extracted with ten times as much 10 per cent salt solution for 24 hours at approximately 4 to 10°C. The extract was filtered and pressed through three thicknesses of clean muslin. The residue was extracted twice again in the same manner except that only eight times as much salt solution was used. The mixed filtrates which were allowed to settle were filtered again through paper pulp on a Buchner funnel and yielded an opalescent grayish lavender solution.

Two methods were used for obtaining the globulin from this filtrate. By the first method, the solution was dialyzed in large parchment paper bags under running tap water for 6 or 7 days, the globulin precipitating in a layer on the bottom of the bags. It was removed to a filter, washed with distilled water, then 95 per cent alcohol. It was suspended in absolute alcohol and in absolute ether respectively for 12 hours, filtered, and partly dried in warmed air currents (below 60°) before finishing in a desiccator over sulfuric acid. By the second method, the salt solution of globulin was made six-tenths saturated with ammonium sulfate and allowed to stand over night in a refrigerator. The precipitated globulin was transferred to filters. It was redissolved in salt

solution, filtered again, and then dialyzed under running tap water 5 days and under distilled water 1 day. The globulin was transferred to hardened filter papers and washed with alcohol. It was suspended as usual in absolute alcohol, then absolute ether, and dried in a vacuum desiccator over sulfuric acid.

The yield of globulin was approximately 19 to 20 per cent of the fat-free meal and contained 18.8 per cent total nitrogen as determined by the Kjeldahl method. It was necessary to powder the globulin as it dried in hard lumps or layers. When dry, the color was a very light gray lavender which had a tendency to fade when heated or upon long standing in contact with air.

TABLE 1

Showing distribution of nitrogen in walnut globulin

	N	TOTAL N
	mgm.	*per cent*
Cystine N	4.95	0.86
Amide N	56.40	9.99
Humin N	10.37	1.84
Arginine N	221.84	39.13
Histidine N	32.32	5.74
Lysine N	59.87	10.61
Mono Amino N	171.33	30.36
Non Amino N	4.94	0.88
Total N	562.02	99.41

Distribution of nitrogen in walnut globulin. The Van Slyke method (6), (7), (8) for determining the nitrogen partition was used on globulin obtained by the second method previously described, after hydrolysis with 20 per cent hydrochloric acid. Three grams hydrolyzed globulin yielded 564.26 mgm. nitrogen. The results of the nitrogen partition are given in table 1.

Feeding experiments with walnut globulin as sole source of protein. Five mice were fed a diet in which the isolated globulin was the sole source of dietary protein. They were fed from weaning for a period of 125 days at an 18 per cent level of protein intake.

Walnut globulin (18.8 per cent N)	18
Salts (Osborne and Mendel)	4
Baked starch	56
Agar	2
Crisco	10
Butterfat	10
Dried brewery yeast	100 mgm. daily

The animals ate heartily and grew normally.

Three mice were fed similarly, but the level of protein intake was reduced to 12 per cent and the baked starch increased to 62 per cent. The animals grew normally.

Three mice were fed similarly with the level of protein intake reduced to 9 per cent and the starch increased to 66 per cent. All the animals

TABLE 2

Results of feeding experiments with walnut proteins

PROTEIN TESTED	NUMBER OF ANIMALS	LENGTH OF EXPERIMENT	AVERAGE TOTAL FOOD INTAKE	PROTEIN INTAKE	AVERAGE GAIN IN BODY WEIGHT	AVERAGE GAIN IN BODY WEIGHT PER GRAM OF PROTEIN	RESULTS
		days	*grams*	*per cent*	*grams*		
Mixed walnut proteins	Rats 2	125	1537.50	18	214.0	0.77	Normal growth
	Mice 3		356.33		14.0	0.22	
	Rats 3	125	1415.0	12	221.00	1.31	Normal growth
	Mice 2		363.5		16.35	0.44	
	Rats 4	125	1316.5	9	163.5	1.39	Mice normal
	Mice 2		345.0		14.4	0.46	Rats minimum limits for normal growth or below
Walnut globulin	Mice 5	125	368	18	18.84	0.28	Normal growth
	Mice 3	125	328	12	16.53	0.42	Normal growth
	Mice 3	125	332	9	14.94	0.50	Minimum limits for normal growth or below
Washed walnut residue proteins remaining after globulin extraction	Rats 4	105	934.25	18	126.0	0.75	Almost normal growth. Many cases of alimentary disturbances due to character of the residue probably
	Mice 3	105	374.3	18	16.4	0.22	

appeared normal. One mouse was entirely comparable with the controls. The other two mice were somewhat below the control animals and were no longer gaining when the work was discontinued. The author feels that a 12 per cent level of intake would be necessary for most mice for normal development.

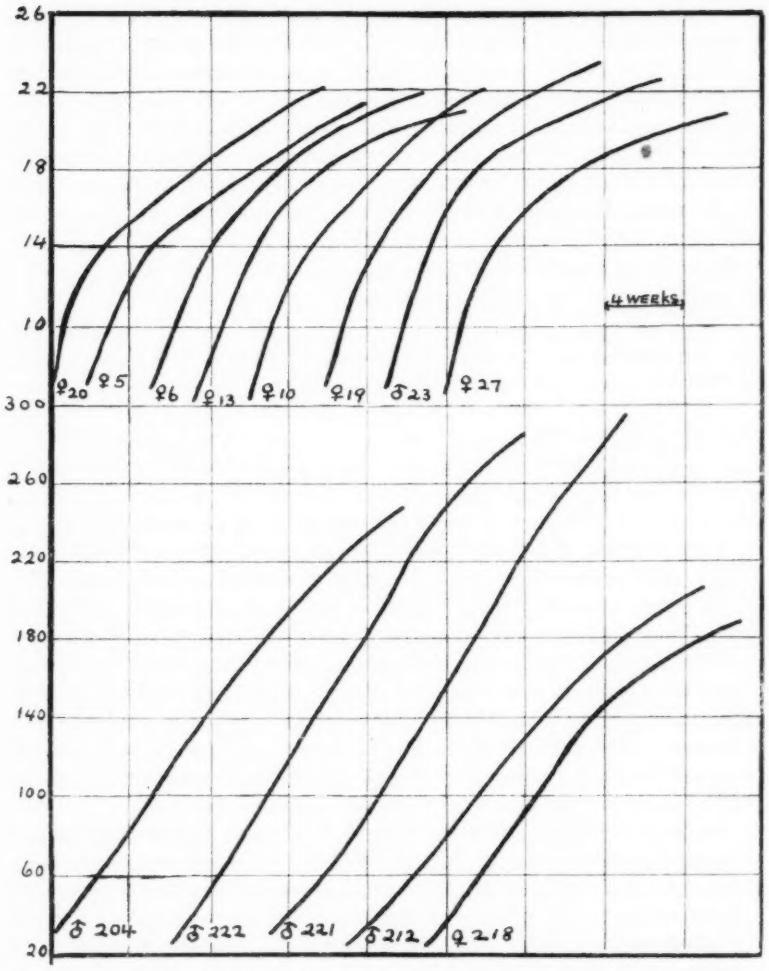

Chart 1. Illustrative growth curves of rats and mice deriving sole dietary protein from the English walnut.

Mice: control—*20*, 18 per cent casein.

Mixed walnut proteins—*5*, 18 per cent intake; *6*, 12 per cent intake; *13*, 9 per cent intake.

Isolated globulin—*19*, 18 per cent intake; *23*, 12 per cent intake; *27*, 9 per cent intake.

Residue proteins after globulin extraction—*10*, 18 per cent intake.

Rats: control—*204*, 18 per cent casein.

Mixed walnut proteins—*222*, 18 per cent intake; *221*, 12 per cent intake; *212*, 9 per cent intake.

Residue proteins after globulin extraction—*210*, 18 per cent intake.

3. The residue from the salt solution extracted walnut meal as sole source of protein. The residue, left after the extraction of globulin from fat-free walnut meal by 10 per cent salt solution, was dialyzed in double muslin bags under running water for 48 hours to remove the sodium chloride. It was then dried in warmed air currents below 60° and ground in a coffee mill. The resulting powder was of a brownish color with a nitrogen content of 4.16 per cent (protein 26 per cent) as determined by the Kjeldahl method. Because of the small amounts obtained, only one level of protein intake (18 per cent) was used. The diet was made up as follows:

Walnut residue (4.16 per cent N)	70
Salts (Osborne and Mendel)	3
Baked starch	7
Crisco	10
Butterfat	10
Dried brewery yeast,	
Rats	400 mgm. daily
Mice	100 mgm. daily

Agar was omitted as the diet possessed laxative properties.

Four rats and three mice were fed from weaning for a period of 125 days. The animals ate the diet rather well after a few days. The mice grew normally, but the rats showed considerable individual variation. One rat was comparable with the controls. The others grew only fairly well, and autopsies showed that in half the cases alimentary disturbances were present. It is probable that the protein present was of good quality but that, over long periods, the unknown carbohydrates and other substances present in the walnut residue resulted in disturbances in many of the animals with a consequent loss of appetite and therefore a growth below the normal.

The biological value of these various proteins of the English walnut is summarized in table 2. It will be noted that excellent consistency is maintained in the results for each of the three levels of intake with each fraction tested when growth in body weight per gram of protein ingested is calculated. The growth curves of type animals fed these various proteins are shown in chart 1.

SUMMARY OF PROTEIN EXPERIMENTS

1. The growth of rats and mice has been observed in a series of feeding experiments in which various fractions of the English walnut were fed as the sole source of protein in an otherwise adequate diet.

a. The mixed walnut proteins obtained by removing the oil with U. S. P. ether when fed at levels of 18 per cent and 12 per cent protein intake, sufficed for normal growth of both rats and mice. Mice grew

normally when the intake of this protein was reduced to 9 per cent, but the growth curves of rats fed at this level indicated development somewhat below level in most cases.

b. The isolated globulin of the English walnut, when fed as the sole dietary protein at 18 and 12 per cent levels of intake, produced normal growth in white mice. However, when the intake of this isolated globulin was reduced to a 9 per cent level, growth was a little below normal, or at least approached the lower limits given for normal growth.

c. The residue left after the fat-free walnut meal had been extracted with salt solution to remove the globulin, when fed to rats and mice as the sole source of dietary protein at an 18 per cent level of intake, permitted almost normal growth. When fed for a long period of time, however, there was evidence that unknown substances in the residue produced alimentary disturbances which interfered with the normal development of most animals.

2. The tannin of the walnut has been found to be a limiting factor in the extraction of walnut globulin and, when present in large quantities in the diet of rats or mice, to be a limiting factor in the normal development of these animals.

3. The nitrogen partition of the chief globulin of the English walnut has been determined by the Van Slyke method after hydrolysis with 20 per cent hydrochloric acid.

THE VITAMIN A CONTENT OF THE ENGLISH WALNUT. The vitamin A content of various nuts including the English walnut was studied by Coward and Drummond (9). They fed 1-gram portions daily to rats which had remained stationary in weight for two weeks upon a diet deficient in vitamin A. They concluded that the nuts examined, although containing large percentages of fat, possess a relatively low food value as sources of vitamin A.

Since the vitamin A content of walnuts had not yet been thoroughly investigated, and since a large excess of walnut oil was obtained in the preparation of the walnut fractions used in the protein study, a series of experiments was planned by the author to study the value of walnut oil as a sole source of vitamin A for rats, using the methods described by Morgan (10). More than sixty animals were used in the experiments which later developed into a study of the value of various parts of the nut as well as various types of extractions of lipoid content. The method of extracting the vitamin is of interest because of the fact that many investigators have found that fat solvents in general often fail to extract this vitamin efficiently from its vegetable sources. In the case of researches upon foods low in vitamin content, it is necessary to find a solvent which will provide a sufficient concentration in order that the remainder of the diet may be kept adequate.

1. Feeding experiments with the whole walnut. The walnut, after blanching, was fed either in whole pieces or ground finely in a meat grinder depending upon which method insured the consumption of the nut. Sometimes it was found necessary to alternate the methods. The nut being about two-thirds fat, and therefore concentrated, the food intake of rats on this diet was always less by weight than that of animals on basal diets. For this reason, if more than 1 gram of nut was fed daily, the nut was ground and mixed with an adequate supply of salts, carbohydrate, agar and protein. The amounts of these ingredients varied with the amount of nut which the animal was made to consume daily. It was not always possible to give the animals a diet as thoroughly balanced as the basal ration. None of the animals were able to consume more than an amount of diet holding approximately 6 grams of nut. In some cases the animals could not be induced to eat this amount. With very young rats, 2 grams was found to be the usual limit and more had to be added gradually. Vitamin A free basal diet was always given together with a 400 mgm. daily yeast allowance. In cases of rats fed 6 grams of nut daily, this was seldom touched. The nut itself has been shown to contain sufficient vitamin B for normal growth of rats (3).

Curative method: Two groups of animals were used. Group I consisted of four rats fed the usual laboratory vitamin A free basal diet until definite symptoms of vitamin A deficiency appeared. One gram of blanched nut added daily sufficed to cure the eye disease and to promote very slight growth for periods varying from 5 to 10 weeks when the allowance was increased to 3 grams daily. This resulted in a greater rise in the growth curve in some cases, but the improvement was not lasting. One rat, continued on 3 grams, died when 175 days old, the autopsy revealing renal calculi. The others were given 6 grams daily which resulted in slow growth below normal, the fur became much improved especially as to thickness, and the animals appeared better in proportion to their ability to consume the whole day's allowance. Autopsies at the close of the experiment revealed the fact that the teeth had been broken and brittle on the lower levels of vitamin, but had grown out approaching normality on the 6-gram ration. Group II consisted of two rats which were treated similarly except that when definite symptoms of deficiency appeared, they were given 2 grams daily. They showed growth curves somewhat better than those on the 1-gram allowance but finally had to receive the necessary increase to 6 grams which induced growth below normal, or a change to butterfat which induced normal growth.

Protective method: Two rats were given 2 grams of whole nut daily in addition to the basal diet from weaning. The allowance was

increased as soon as possible. Not until a 6-gram allowance was reached did growth occur and this was at a rate below normal.

Conclusion: The whole walnut contains vitamin A in so small amounts that not enough can be consumed by rats in addition to the other necessary nutrients to induce normal development.

2. Feeding experiments with cold pressed walnut oil as sole source of vitamin A. The oil obtained by pressing ground blanched nuts in a tincture press represented about one-third of the contained fat. The oil was prepared fresh weekly and kept tightly stoppered in a refrigerator. The only way by which the animals could be induced to consume it was by incorporating it into the basal diet. Since this nut is low in vitamin A, the entire 20 per cent fat content of the diet was given as cold pressed oil. No more than this could be consumed by the animals.

Curative method: Two rats were fed upon the laboratory vitamin A free basal diet until deficiency symptoms appeared. The crisco of the diet was then replaced by the walnut oil. The animals did not like the diet and ate sparingly. They maintained themselves only for 4 or 5 weeks, declined rapidly, and died.

Protective method: Two rats were fed the 20 per cent cold pressed oil diet from weaning. They grew slowly, below normal, and did not develop eye disease until approximately the 15th week on the diet instead of the 4th or 5th week as is the case on vitamin A free basal diet. When they began declining, they were given the same diet but with 10 per cent of the walnut oil replaced by butterfat. They returned to normal.

Conclusions: Cold pressed walnut oil constituting 20 per cent of the diet does not possess sufficient vitamin A for normal growth of rats, nor does it possess sufficient to cure eye disease or to bring about a return to normal in rats which have declined on a diet deficient in this vitamin. The oil itself is probably not toxic. By replacing only half of the walnut oil used in the diet with butterfat as a source of vitamin A, the animals declining on the oil diet returned to normal.

3. Feeding experiments with ether extracted walnut oil as sole source of vitamin A. The oil used was extracted from ground blanched nuts using U. S. P. ether as solvent. The ether was distilled off over a water bath, and the oil was kept stoppered in a refrigerator. It was prepared weekly and was incorporated as 20 per cent of the diet of the animals in exactly the same manner as was the cold pressed oil.

Curative method: Four rats were fed the usual vitamin A free diet until definite deficiency symptoms appeared. Two of the rats were given no additions to this diet. They died about 3 weeks after the definite symptoms were manifested. The other two rats were given

the 20 per cent ether extracted oil diet. One rat barely maintained itself for a number of weeks, declined rapidly, and died at the age of 126 days. The animal shrank in size, and the tail bone joints were plainly visible for days. The fur was rough and the eyes in a bad condition. The animal could not stand upon its feet. An autopsy showed broken and defective teeth; infected left kidney with pus; costa-chondral junctions hemorrhagic, enlarged and beaded. The other rat grew slowly, at a rate not much above maintenance, for about 10 weeks. Half of the walnut oil of the diet was replaced by butter fat and this diet was continued 11 weeks. An autopsy showed the teeth growing out, and the fur in a much improved condition. The animal was returning to normal.

Protective method: Three rats were fed the 20 per cent ether extracted oil diet from weaning. They developed definite symptoms of vitamin A deficiency at 8, 12 and 14 weeks respectively as compared with the average 4 weeks on vitamin A free basal diet. Their fur was very rough and unkempt. Upon replacing half of the walnut oil with butterfat, the animals returned to normal.

Conclusions: Practically no difference exists in the amount of vitamin A present in walnut oil, whether cold-pressed or ether extracted. When either of these constitutes 20 per cent of the diet of rats, not enough vitamin is present to restore rats declining from lack of vitamin A. The vitamin A content of walnuts must be present either in amounts too low to provide sufficient for normal growth of rats or in such association in the nut that it is not removed with the oil either by pressure or ether extraction.

4. Feeding experiments with walnut meal as sole source of vitamin A. The walnut meal used was that left after the extraction of fat from blanched ground nuts by U. S. P. ether and was the same as that used in the feeding experiments on mixed walnut proteins. The ether extract did not contain any considerable amount of vitamin A, and it was thought worth while to determine whether or not this was due to a lack of vitamin or to the failure of its extraction by ether. One gram of walnut meal represents the non-fat content of 3 grams of walnut. It must be remembered that at least some vitamin A was removed by ether as shown by the fact that eye disease was postponed for 8 or 10 weeks when ether extracted oil was used as a sole source of vitamin A.

Curative method: Three rats were fed the vitamin A free basal diet until definite deficiency symptoms appeared. Then 1 gram of meal was placed on top of the basal diet daily. All the rats ate the meal. They grew at a rate very much below normal, but the eye disease was cured in all cases. One rat died when 135 days old. The autopsy showed front teeth and jaw bones very fragile; bladder distended; spleen much

enlarged and congested. The other rats were raised to a 2-gram allowance. They grew somewhat better for a number of weeks, when butterfat was added to the diet. The one which did not return to normal showed renal calculi at the autopsy.

Protective method: Three rats were given 1 gram of walnut meal from weaning. They grew somewhat below normal for about 15 weeks. One was killed and autopsied when 135 days old. The animal was normal except that the bones were perhaps somewhat easier to break than those of control animals. The other rats were given a 2-gram allowance. This produced slow growth below normal. The amount could not be further increased because of lack of meal. The animals upon autopsy appeared normal except as to weight. The bones and teeth were also normal.

Conclusions: The conclusions drawn are limited because of the short period of the experiment and the limited amount of meal used. It would appear that walnut meal residue left after ether extraction of walnut oil contains vitamin A in such amount that a 2-gram daily allowance does not suffice to restore rats declining on a diet deficient in this vitamin, but does protect the animals to some extent if fed from weaning. It is probable that a diet could be planned using this meal as a sole source of vitamin A which would produce normal development.

The fact that better growth results are obtained with residues left after ether extraction of walnuts than with the ether extract itself indicates the slight solvent power of ether for the vitamin A of walnuts. The vitamin appears to be associated chiefly with the non-fat fraction of the nut, and it is not removed with the oil by pressure or by the usual fat solvents.

It was observed in the series of experiments that walnut meal in amounts of 1 gram or more daily as a sole source of vitamin A prevented or cured eye disease in 100 per cent of the cases. It was further observed that an amount of whole nut containing an equivalent amount of this meal (approximately 3 grams of nut) also prevented or cured eye disease.

5. Feeding experiments with hot alcohol extract of walnuts as sole source of vitamin A. The extract for this experiment was prepared as follows: 300 grams of blanched and finely ground walnuts were extracted for one hour with 95 per cent alcohol over a water bath, using a reflux condenser. The material was strained while hot through two thicknesses of a good quality of clean cheese-cloth into a distilling flask, and the alcohol was distilled off over a water bath. The yield averaged 100 grams of extract, representing about one-half of the oil present in the nuts used.

The oily extract was incorporated as 5, 10 and 20 per cent of the diet respectively. Three rats were fed the 5 per cent alcohol extract diet from weaning. They developed vitamin A deficiency symptoms in as short a period as those on vitamin A free basal diet. They ate very

TABLE 3

Results of feeding experiments to determine vitamin A content of walnuts

PART OF NUT USED	METHOD USED	NUMBER OF RATS	LENGTH OF EXPERIMENT	AMOUNT IN THE DIET	RESULTS
			days		
Whole blanched walnut	Curative	6	175	6 gms. daily	No eye disease. Growth below normal
	Protective	2			
Cold pressed walnut oil	Curative	2	140	20 per cent of diet	Decline and death
	Protective	2			Eye disease and decline postponed 9 or 10 weeks
Ether extracted walnut oil	Curative	4	150	20 per cent of diet	Decline and death
	Protective	3			Eye disease and decline postponed 8 or 9 weeks
Fat-free walnut meal	Curative	3	150	2 gms. daily	Cured eye disease. Growth not brought to normal
	Protective	3			No eye disease. Protects to considerable extent
Hot alcohol extract of blanched walnuts	Protective	3	150	5 per cent of diet	No protection
		2		10 per cent of diet	No protection
		3		20 per cent of diet	Normal development

sparingly. They were at once transferred to the 20 per cent alcohol extract diet but did not return to normal until 10 per cent of the extract was replaced by butterfat. Those not receiving butterfat showed upon autopsy renal calculi and kidney infection.

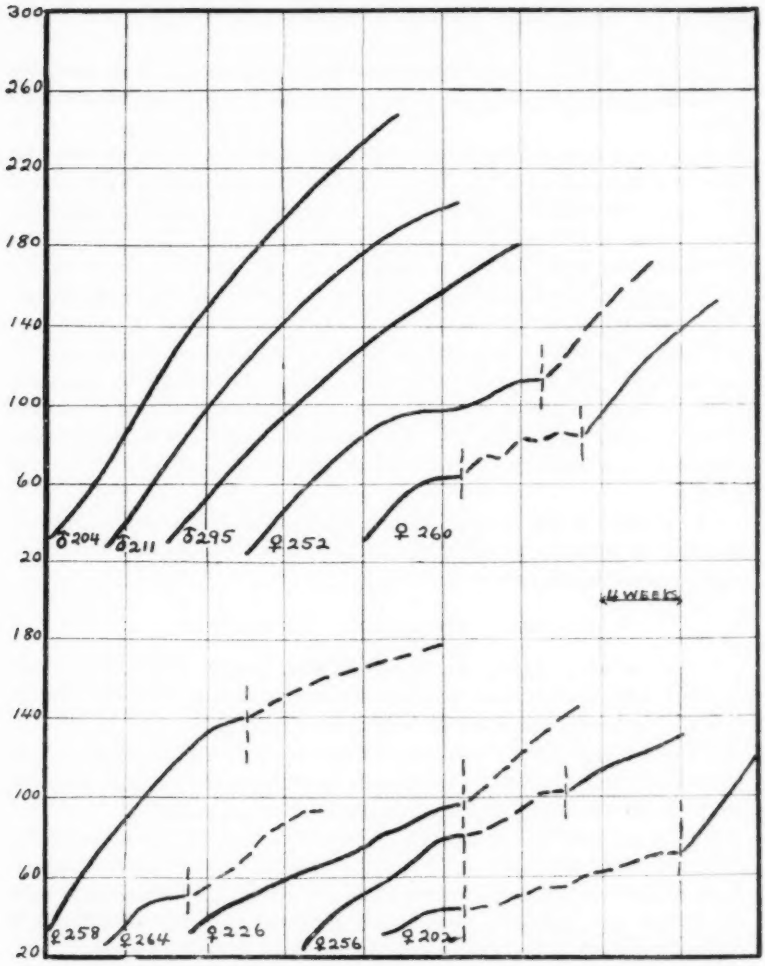

Chart 2. Illustrative growth curves of rats deriving sole dietary vitamin A from the English walnut. Protective method.

Controls—*204* and *211*, 10 per cent butterfat.

Whole blanched walnut—*256*, 2 grams daily to first dotted line, 3 grams daily to second line, 6 grams daily; *202*, 1 gram daily to first line, 3 grams daily to second, 6 grams daily.

Cold pressed walnut oil—*226*, 20 per cent of diet to dotted line, 10 per cent butterfat.

Ether extracted walnut oil—*252*, 20 per cent of diet to dotted line, 10 per cent butterfat.

Alcohol extract of walnuts—*264*, 5 per cent of diet to first dotted line, 20 per cent of diet, death; *260*, 10 per cent of diet to first line, 20 per cent to second, 10 per cent butterfat; *295*, 20 per cent of diet.

Fat-free walnut meal—*258*, 1 gram daily to dotted line, then 2 grams daily.

Two rats were fed the 10 per cent alcohol extract diet from weaning. The results were the same as in the case of the 5 per cent alcohol extract diet.

Three rats were fed the 20 per cent alcohol extract diet from weaning. All developed normally, had good coats of fur, and were very active. When 150 days old they were killed and autopsied, and found normal. They ate as much as 12 to 15 grams of diet daily. This represented the alcohol extract of at least 9 grams of nut. Each 10 grams of diet held the extract of 6 grams of nut so that really large amounts of nuts were represented in the diets.

Conclusions: The vitamin A content of walnuts is extractable by means of hot alcohol, but not to any appreciable extent by means of pressure or by means of U. S. P. ether. A hot alcohol extract of walnuts when constituting 20 per cent of an otherwise adequate diet, possesses sufficient vitamin A to support normal growth of rats for at least 150 days.

The results of the feeding experiments to determine the vitamin A content of walnuts are summarized in table 3. Growth curves of typical animals are shown in chart 2.

SUMMARY OF VITAMIN A EXPERIMENTS

1. The vitamin A content of the English walnut has been investigated in a series of feeding experiments on rats, using both the curative and the protective methods of testing for vitamins.

2. It has been found that the whole walnut contains vitamin A in so small amounts that not enough can be consumed by rats in addition to the other necessary nutrients to furnish normal growth.

3. The vitamin A of walnuts is not obtained to any appreciable extent with the oil which is extracted by pressure or by U. S. P. ether. It is, however, obtained by extracting the ground blanched nuts with hot 95 per cent alcohol. Such an extract contains sufficient vitamin A to produce normal growth in rats for at least 150 days when it constitutes 20 per cent of the diet as the sole source of both fat and vitamin A.

4. The decline of rats fed a diet containing 20 per cent of cold-pressed or ether extracted walnut oil as the sole source of both fat and vitamin A is probably not due to any toxic properties possessed by the oil. By replacing one-half of the oil with butterfat, animals declining from vitamin A deficiency on this diet are restored to normal.

5. It has been noted that in many cases of vitamin A deficiency in rats, phosphatic renal calculi and kidney infection are present. This is the probable cause of the failure of these animals to return to normal even after additions of highly adequate sources of vitamin A to the diet.

BIBLIOGRAPHY

(1) Osborne and Harris: Journ. Amer. Chem. Soc., 1903, xxv, 853.
(2) Cajori: Journ. Biol. Chem., 1920, xliii, 583.
(3) Cajori: Journ. Biol. Chem., 1921, xlix, 389.
(4) Schmidt: Journ. Ind. and Eng. Chem., 1916, viii, 165.
(5) Johns, Finks and Paul: Journ. Biol. Chem., 1919, xxxvii, 497.
(6) Van Slyke: Journ. Biol. Chem., 1912–12, x, 15.
(7) Van Slyke: Journ. Biol. Chem., 1915, xxii, 281.
(8) Plimmer: The chemical constitution of the proteins. New York, 1917.
(9) Coward and Drummond: Biochem. Journ., 1920, xiv, 665.
(10) Morgan: This Journal, 1923, lxiv, 522.

ON THE SOURCES OF HEAT IN MUSCLE CONTRACTION

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In a former note (1) it was shown that if one allows for possible heat of elastic origin and adds this to the probable heat of chemical origin, the sum thus obtained agrees exactly with the systolic heat observed in the terrapin's thermocardiogram (2). It becomes therefore of further interest to inquire whether the heat observed by Hartree and Hill (3), (4) for the period of rising tension of frog's sartorius muscle likewise may include heat of elastic origin.

Before attempting to answer this it is necessary to have good reason to believe that muscle in isometric contraction undergoes elastic strain, for doubtless the method used by those authors was the one described in their earlier work where the relaxed muscle is stretched between and fixed at two immovable points. Now it would seem, as those authors argue, that development of tension under such conditions would not give rise to an elastic strain; and that, as they specifically state, heat of elastic origin may be obviated by working with rigidly isometric contractions (5 p. 172). However, further inquiry as to what really takes place within a muscle during isometric contraction will, I think, lead to a directly opposite view. For may it not be that following directly upon stimulation a muscle whose ends are rigidly fixed at the "relaxed" distance suddenly finds itself a shorter elastic body, and therefore *only reaches between the two original fixed points because it undergoes elastic stretching?*[1] If this be the case then in the experiments on sartorius muscles there must have been also heat of elastic origin which should be substracted first in order to get at the probable proportion of heat of chemical origin.

Consider that this latter assumption is a valid one; would the excess of initial heat found in sartorius come within the limits at all of a

[1] This idea is probably not new to physiologists. It is clear Prof. A. V. Hill had it in mind at an earlier time, for example, when he says (Journ. Physiol., 1913, xlvi, 467), "(b) that after maximum [isometric] tension has developed the muscle is merely a new elastic body. . . ." Later papers, however (3), (4), (5) seem to preclude from the discussion the possibility of a thermo-elastic effect during development of isometric tension.

possible thermo-elastic effect? It appears from the experiments of Hartree and Hill (4) that for "shortest tetanus" (0.03 sec.) frog's double sartorius muscles at 20°C. produce an initial (anoxidative) heat of about 0.0074 calorie per gram muscle, and that the ratio of the heat of recovery (oxidative phase) to this initial heat, "Hr/Ho," is 1.4. The total heat of the muscle action must therefore be put at about (1.4 × 0.0074) + 0.0074, or 0.01776 calorie per gram muscle. These muscles were stimulated directly by an electric current which produces say 2 per cent of the initial heat, an item also that may be corrected for.

If we allow X to represent the heat of elastic origin, and equate the ratio of the heats observed with the ratio of those demanded by the chemical reactions (see (1, p. 584) for this ratio), we have

$$\frac{(0.0074 - 0.000148) - X}{(0.017760 - 0.000148) - X} = \frac{1}{4.42}$$

whence $X = 0.00422$ calorie.

Here the heat of elastic origin then would be about 23 per cent of the total heat observed. While this seems excessive it is not an improbable amount if it is true as the authors have stated elsewhere (5, p. 171) that the thermo-elastic heat may be "a fifth to a half of the rise produced by a strong twitch."

Taking 0.00422 calorie as heat of elastic origin for the moment, it appears that the tension per gram muscle required to produce it may be as much as 0.00422 (2.4×10^{-6}), or nearly ~~1770~~ 760 grams.[2] The weights of the muscles in these experiments are not given but in his *Ergebnisse* article, p. 446, A. V. Hill reports that in one series of experiments the weights of frog's double sartorius muscles varied from 0.13 to 0.24 grams. The total tension exerted by these muscles varied from 32 to 76 grams. Doi (6) gives 82 grams as a highest "total tension sustained" by sartorius in response to a single electric shock, but gives no weights of the muscles. Using the most favorable data then, the total tension exerted by sartorius muscle in a single twitch may have been as much as 630 grams, $\frac{1}{0.13} \times 82$.

In the experiments under consideration the muscles however were not responding in single twitches but in "short lasting tetani" of 0.03 second, the stimulating current being "an alternating current of about 25 volts and 90 periods per second" (3, p. 96). This then admits of a probable 3-twitch response, that according to Fick would triple the heat production and also, one assumes, the thermo-elastic effect. If so then the figure representing the equivalent of this effect in grams

[2] For the factor, 2.4×10^{-6} and the other data in this calculation see (1, p. 586) and (2, p. 283).

ought to be no more than three times greater than that representing the tension in grams exerted by a single twitch, that is, not more than 3×630, or 1890 grams. As shown in the above calculation, the thermo-elastic effect in Hill and Hartree's experiments may have been as much as ~~1770~~ 1760 grams.

It therefore appears not improbable in the case of the sartorius muscle developing tension isometrically (in a rigidly fixed position) that there is a thermo-elastic effect at hand of considerable magnitude. Obviously a thermo-elastic effect obtaining under the conditions of the isometric twitch is one that can not be demonstrated experimentally; the closeness with which the experimental facts of the case fit in with the hypothesis however would indicate that it is a factor that can not be left out of account.

BIBLIOGRAPHY

(1) SNYDER: This Journal, 1923, lxiii, 583.
(2) SNYDER: This Journal, 1922, lix, 282.
(3) HILL AND HARTREE: Jour. of Physiol., 1920, liv, 84.
(4) HARTREE AND HILL: Ibid., 1922, lvi, 367.
(5) HILL AND HARTREE: Phil. Trans. Roy. Soc. London, 1921, B, ccx, 153.
(6) DOI: Journ. Physiol., 1920, liv, 226.

THE AVERAGE EPINEPHRIN OUTPUT IN CATS AND DOGS

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Several years ago we published tables showing the epinephrin outputs estimated by the rabbit intestine segment method in 45 cats and 17 dogs (1) (2).[1] The average output per kilogram per minute for the 16 etherized cats was given as 0.0002 mgm. and for the urethanized cats 0.00025 mgm. For the 45 cats the average was 0.00023 mgm. per minute. For 17 dogs, anesthetized with morphine and ether or with ether alone, the average output per kilogram per minute was given as 0.00022 mgm.

In these tables the outputs are stated as far as possible in round numbers; for example, an output which would be calculated as 0.00021 or 0.00019 mgm. per kgm. per minute is given as 0.0002. This was done to avoid giving the impression that the second significant figure can always be accurately obtained from an assay by rabbit intestine segments. This is not the case, much depending upon the sensitiveness and constancy of the reactions given by the segment, something upon the magnitude of the output. We have therefore thought it worth while to calculate again to the second significant figure the results in the previously published tables for the purpose of deducing an average output. For the 45 cats the average comes out 0.000230 mgm. per kgm. per minute, the same as before. If we omit one cat (no. 65) which was anesthetized by increased intracranial pressure, ether being used only in the preliminary operation, the average for 44 cats, under urethane or ether, is 0.000225 mgm. per kgm. per minute. For 28 urethanized cats the average is 0.000241 mgm. per kgm. per minute, and for 16 etherized cats 0.000198 mgm.

[1] We take the opportunity of making one or two small corrections in tables 1 and 3 of the second paper. For dog 15 the epinephrin concentration in the blood samples should be 1:5,000,000 and 1:4,300,000 (the concentrations in the serum were given inadvertently) and the outputs per minute for the animal 0.0016 and 0.0017 mgm. For cat 287 the output for the animal should be 0.00025 and the output per kgm. per minute 0.00007 mgm. For cat 295 the concentration should be 1:8,000,000, the output for the animal 0.0004 and the output per kgm. per minute 0.00014 mgm. For cat 211 the output per kgm. per minute should be 0.00015 mgm.

TABLE 1

NUMBER OF ANIMAL	SEX	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN		ANESTHETIC
				Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute	
		kgm.	*grams*						*mgm.*	*mgm.*	
14	F	2.58	0.328	10.3	5		2.06	1: 3,500,000	0.00059	0.00023	U
308	M	3.33	0.473	6.7	2		3.35	1: 7,000,000	0.00048	0.00014	E
311	M	3.89	0.668	7.1	1	30	4.73	1: 7,800,000	0.00060	0.00015	E
312	F	2.80	0.340	4.3	3		1.43	1: 4,000,000	0.00036	0.00013	E
317	F	2.00	0.290	7.65	2		3.82	1: 9,000,000	0.00042	0.00021	E
319	M	2.30	0.301	5.1	3		1.70	1: 5,750,000	0.00030	0.00013	E
322	F	2.25	0.487	7.0	2		3.50	1: 4,500,000	0.00078	0.00035	E
324	F	2.10	0.367	5.55	3		1.85	1: 9,000,000	0.00021	0.00010	E
325	M	2.05	0.252	5.15	3		1.72	1: 2,500,000	0.00069	0.00034	E
356	M	2.00	0.545	6.3	3		2.10	1: 2,000,000	0.00105	0.00050	Local
357	F	2.50	0.602	5.35	4		1.34	1: 3,500,000	0.00038	0.00015	Local
361	F	2.65	0.385	7.15	3		2.38	1: 1,330,000	0.0018	0.00067	Local
376	F	2.16	0.472	4.15	4		1.04	1: 5,700,000	0.00018	0.00008	U
377	F	2.70	0.367	9.95	1	30	6.63	1: 6,000,000	0.0011	0.00041	E
378	M	3.07	0.518	5.35	2		2.67	1: 4,500,000	0.00059	0.00019	U
381	M	2.22	0.269	6.35	5		1.27	1: 2,800,000	0.00045	0.00020	U
383	M	3.98	0.602	8.05	1	30	5.36	1: 5,400,000	0.0010	0.00025	U
384	M	2.63	0.275	4.95	2		2.47	1: 3,500,000	0.00070	0.00027	U
385	M	2.75	0.612	6.2	3		2.06	1: 2,900,000	0.00071	0.00025	E
399	F	2.69	0.268	5.3	1	30	3.53	1: 5,000,000	0.00071	0.00026	E
406	F	2.09	0.474	3.75	2		1.87	1: 3,600,000	0.00052	0.00025	E
407	F	2.46	0.375	4.85	1	30	3.23	1: 6,000,000	0.00054	0.00022	E
415	F	2.80	0.373	5.75	2		2.87	1: 3,500,000	0.00082	0.00029	E
417	F	2.26	0.561	3.35	2		1.67	1: 5,700,000	0.00029	0.00013	U

418	F	1.96	0.232	3.2	1	30	2.13	1: 4,000,000	0.00052	0.00026	E
431	F	2.05	0.259	7.0	2		3.5	1: 7,500,000	0.00047	0.00023	E
432	M	3.67		6.5	1		6.5	1:12,300,000	0.00053	0.00014	E
435	F	2.50	0.314	1.9	2	30	0.76	1: 800,000	0.00095	0.00038	E
458	F	2.21	0.395	3.7	2		1.85	1: 3,500,000	0.00053	0.00024	E
459	F	2.92	0.302	3.6	3		1.20	1: 1,600,000	0.00075	0.00026	E
460	F	3.08	0.484	5.4	1	30	3.60	1: 2,800,000	0.00129	0.00042	U
462	F	2.65	0.310	3.65	2		1.82	1: 9,000,000	0.00020	0.00008	E
463	F	2.87	0.345	5.7	1	30	3.80	1: 7,000,000	0.00054	0.00019	E
464	M	2.85	0.518	4.53	2		2.27	1: 5,500,000	0.00041	0.00014	U
465	F	2.86	0.405	4.25	2		2.12	1: 4,300,000	0.00049	0.00018	E
466	F	2.77	0.277	5.65	1	30	3.76	1: 9,500,000	0.0004	0.00014	E
467	F	2.43	0.502	3.9	3		1.30	1: 5,500,000	0.00024	0.00010	E
468	F	3.42	0.562	4.7	1	30	3.13	1: 6,000,000	0.00052	0.00015	E
469	F	3.30	0.399	6.6	1	30	4.4	1: 5,000,000	0.00088	0.00026	E
471	F	2.75	0.410	5.37	1		5.37	1:20,000,000	0.00027	0.00010	E
472	M	2.91	0.368	4.4	2		2.20	1: 5,000,000	0.00044	0.00014	E
476	F	2.15	0.332	2.2	3		0.73	1: 2,000,000	0.00036	0.00017	E
479	M	2.55	0.402	4.75	2		2.37	1: 5,000,000	0.00047	0.00018	E
484	F	2.80	0.313	3.75	2		1.87	1: 4,500,000	0.00042	0.00015	E
486	M	4.21	0.714	9.65		45	12.87	1:40,000,000	0.00032	0.00008	E
616	M	2.71	0.460	6.6	1	30	4.40	1:10,000,000	0.00044	0.00016	E
619	M	3.39	0.506	10.0	1	30	6.7	1: 7,000,000	0.00096	0.00028	E
624	F	2.39	0.422	3.4	2		1.7	1: 3,000,000	0.00057	0.00024	U
625	F	2.29	0.305	3.0	2		1.5	1: 3,100,000	0.00048	0.00021	E
626	F	2.16	0.410	5.13	2		2.56	1: 5,000,000	0.00051	0.00024	E
807	F	2.45		5.05	1	30	3.37	1: 3,900,000	0.00086	0.00035	E
808	F	3.17		5.0	2		2.50	1: 3,500,000	0.00071	0.00022	E
809	M	2.68		4.9	2		2.45	1: 8,000,000	0.00031	0.00011	E
810	M	2.00	0.260	3.85	2		1.92	1: 4,000,000	0.00048	0.00024	E
814	F	2.17		6.35	4		1.59	1: 2,500,000	0.00064	0.00029	E

TABLE 1—*Concluded*

NUMBER OF ANIMAL	SEX	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN		ANESTHETIC
				Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute	
		kgm.	*grams*						*mgm.*	*mgm.*	
815	F	2.51	0.237	6.8	3		2.27	1: 1,700,000	0.00133	0.00053	U
816	F	2.02	0.337	3.7	2		1.85	1: 2,500,000	0.00074	0.00037	U
817	M	3.24	0.346	4.3	6		0.72	1: 2,500,000	0.00057*	0.00018*	U
821	F	2.05		5.65	4		1.41	1: 900,000	0.00156	0.00076	U
836	M	2.97	0.281	3.7	3		1.23	1: 3,750,000	0.00033	0.00011	U
840	M	3.46	0.441	3.55	2		1.78	1: 2,500,000	0.00071	0.00020	E
841	F	2.37	0.301	4.2	3		1.40	1: 2,000,000	0.00070	0.00029	E
21	M	10.0	1.05	13.0	1	40	7.8	1: 3,200,000	0.00244	0.00024	E
24	M	14.9	1.15	22.9	3	55	5.8	1: 3,000,000	0.00193	0.00013	E
309	F	8.1	0.942	15.9	1		15.9	1: 6,100,000	0.0026	0.00032	M, E
327	M	7.4	0.615	12.35		30	24.7	1:15,000,000	0.00165	0.00022	M, E
352	M	6.5	1.886	9.4	2		4.7	1: 3,300,000	0.00142	0.00022	M, E
355	F	17.85	2.30	10.9	3		3.63	1: 5,000,000	0.00145*	0.00008*	M, E
379	M	4.8	0.894	8.15	1	30	5.43	1: 3,000,000	0.00181	0.00038	M, E
380	M	5.5	1.082	9.7	1		9.7	1: 4,500,000	0.00215	0.00039	M, E
382	M	7.2	0.870	10.95	1	30	7.3	1: 4,000,000	0.00182	0.00025	M, E
487	M	13.78	1.70	10.9	3		3.63	1: 2,300,000	0.00316*	0.00023*	E
620	M	6.4	1.192	8.2	1		8.2	1: 4,000,000	0.00205	0.00032	E
621	F	7.0	1.712	8.25	1		8.25	1: 5,000,000	0.00165	0.00024	E
622	M	4.8	0.87	4.58	1		4.58	1: 4,700,000	0.00097	0.00020	E
623	M	6.6	0.82	9.15	1		9.15	1: 4,200,000	0.00218	0.00033	E
680	F	5.83	1.046	8.0	1	30	5.33	1:13,000,000	0.00041	0.00007	E

* In cats 817 and dogs 355 and 487 the output measured was that from one adrenal. The numbers have been doubled in the second and third columns from the right of the table.

The last 15 animals in the table are dogs, the rest cats. Cats 435, 458, 462, 468, 471 and 624 were in advanced pregnancy. Cat 381 had received no food for 3 days.

We are now in possession of additional estimations on 59 cats anesthetized with ether or urethane, and on 15 dogs anesthetized with ether or with morphine and ether; and are therefore in a position to see whether the method gives results which agree in these two large series of experiments, the first belonging to the years 1917 to 1919, and the second to the years 1919 to 1923. The essential data on the new series of animals are given in table 1. Three cats whose epinephrin output was estimated under local anesthesia are included in the table, but are not utilized in calculating the average. For the purpose of obtaining the average, the outputs per kilogram per minute are calculated to the second significant figure. The average for the 59 cats of the new series is 0.000227 mgm. per kgm. per minute, the same as for the previous series. For the 15 urethanized cats the average is 0.000273 mgm. per kgm. per minute, and for the 44 etherized cats 0.000211 mgm. Combining the two series we get for 43 cats under urethane an average output of 0.000252 mgm. per kgm. per minute, and for 60 cats under ether 0.000207 mgm.

The average for 103 cats, without reference to the anesthetic, is 0.000226 ± 0.000007 mgm. per kgm. per minute ($\sigma = 0.00010$). If the outputs above 0.00050 mgm. per kgm. per minute (3 cats) and those below 0.00010 mgm. (4 cats) be eliminated, not because there is any doubt about their accuracy but because they lie so far from the mean, we get as the average for 96 cats 0.000220 mgm. per kgm. per minute.

The variations in the individual animals are shown graphically in figure 1. It will be observed that the great majority (84 per cent) of the outputs fall between 0.00030 and 0.00010 mgm. per kgm. per minute. In the group of etherized cats 85 per cent, and in the group of urethanized animals 81 per cent of the outputs lie between these limits.

If the new series of 15 dogs (table 1) be added to the old one, the average output for the 32 dogs (the outputs being calculated, as explained above, to the second significant figure) is 0.000227 ± 0.0000096 mgm. per kgm. per minute ($\sigma = 0.000080$), the same as for the cats. So exact a coincidence, with one of the series containing relatively so small a number as 32 animals, is no doubt accidental. And it must be remarked that we do not know whether, with great variations in the size of the animals (3 or 4 of the dogs were much larger than the rest), the relation of epinephrin output to body weight would be the same. Nevertheless, the fact that the average output per kilogram per minute is practically the same in dogs and cats is clearly revealed. The distribution of the individual results is also strikingly similar, as seen in figure 1. The absence of such extremely high outputs as occasionally occur in the cats may, in the case of the dogs, be related to the much greater body weights, in so far as it is not due simply to the relatively small number of animals.

In figure 1 is further displayed the distribution of the individual outputs for males and females. As it would be extremely improbable that a sexual difference should exist in cats and not in dogs, and as the average

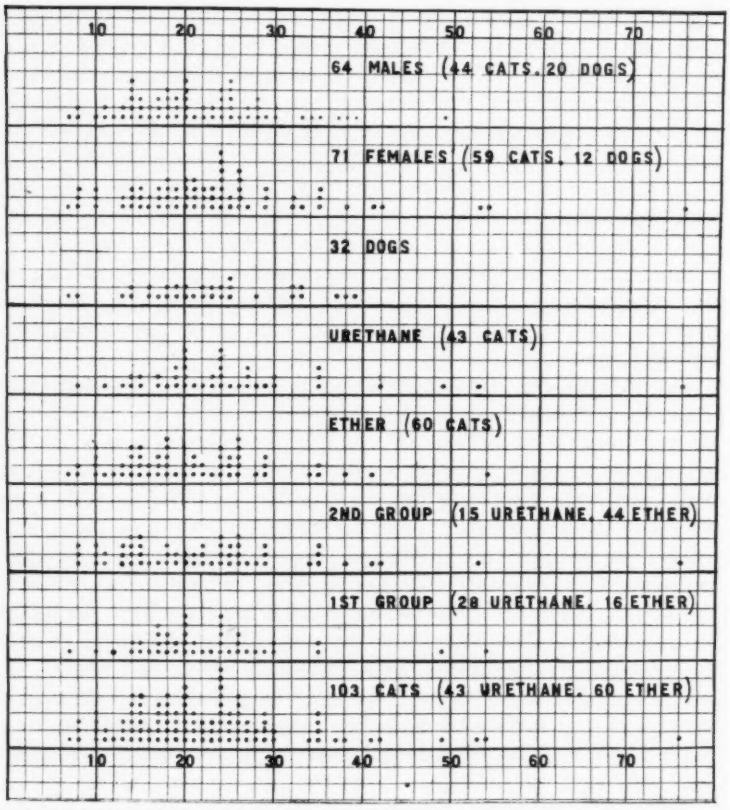

Fig. 1. Chart showing the distribution of the epinephrin outputs, expressed in hundred-thousandths of a milligram of base per kilogram of body weight per minute, in 103 cats and 32 dogs, as determined by the rabbit intestine segment method. Each point represents the output determined in one animal. Where the output was determined on more than one specimen of adrenal vein blood from the same animal the point represents the average of the estimations. For convenience, points on the same vertical line are plotted at uniform distances, but this, of course, has no quantitative significance, except that that height of a vertical row of points is a measure of the class frequency.

epinephrin output per unit of body weight is the same in cats as in dogs, the two groups are combined for this purpose. This gives 71 females and 64 males. The average output for the females is 0.000235 mgm.

per kgm. per minute, and for the males 0.000218 mgm. per kgm. per minute. The distribution of the individual results, as shown in figure 1, is not notably different in the two groups.

If we group the male and female cats in which the same anesthetic was employed, choosing the etherized animals, as forming the largest group, we obtain for the etherized male cats an average output of 0.000174 mgm. per kgm. per minute, and for the etherized females 0.000228 mgm. The apparent excess of output for the females is not due to the inclusion among the etherized animals of 5 cats in advanced pregnancy, since the average output for these was only 0.00019 mgm. per kgm. per minute. The average output for 9 cats in advanced pregnancy (5 anesthetized with ether and 4 with urethane) was 0.00020 mgm. per kgm. per minute. The question arises whether the output for the pregnant animals should be calculated on the gross body weight or on the weight after removal of the embryos. Our calculations are made on the gross weight, although even if the net weight after removal of the embryos is taken, it does not bring the average for pregnant cats above the general average for all the cats. Thus in cat 624 (under urethane) the output calculated on the net weight (2.09 kgm. instead of 2.39 kgm.) is 0.00027 mgm. per kgm. per minute instead of 0.00024, as given in table 1. We do not know whether the apparently larger output in the female cats is related to the greater average body weight of the males, which might perhaps be associated with a smaller output per kilogram of body weight, or is simply due to the number of animals being too small. That the last named factor is involved is indicated by the fact that in the dogs the relation is reversed. For the male dogs the average body weight is 7.48 kgm. and for the female dogs 7.78 kgm. For the male cats the average weight is 3.08 kgm. and for the female cats 2.50 kgm.

If cats 65, 356, 357 and 361 are included with the 103 cats in which a general anesthetic was used, we get for 107 cats an average output of 0.000234 mgm. per kgm. per minute.

The close agreement in the results of our two extensive series of measurements on cats strengthens our opinion that the rabbit intestine segment method, when properly employed, is the best method we possess at present for determining the epinephrin output.

At an earlier stage in this work (1) we thought, because of apparent discrepancies between the average output of epinephrin as determined by auto-assay (especially with the pupil after removal of the superior cervical ganglion), that there might be risk of some loss of epinephrin in defibrinating the blood and in the interval elapsing between its withdrawal from the body and the carrying out of the assay on the segments. At that time we had only made direct comparisons on two animals (1), first determining the output as well as possible by means of the pupil reaction, and then obtaining blood from the adrenal veins and

assaying the epinephrin in it on the rabbit segments. In one of these cats (no. 85), in which a good blood flow was still present when the adrenal samples were being collected, there was a fair agreement between the determinations by the two methods. But in the other (no. 86) the blood flow, which was excellent during the pupil observations (so good that in some of the observations the "long" cava pocket was filled in 15 seconds), had declined so much at the time of collection of the adrenal blood that it is probable that the calculated output was less than the real output, and the deficiency in the output, as determined by the segment assay, was considerable. We soon came to see that the apparent excess in the average output, as determined by the pupil reaction, over that determined by the rabbit segment method was not a real difference, and since then we have employed only the values deduced from the segment assays as the approximately correct values for the concentration of epinephrin in the adrenal vein blood and its rate of output under the conditions of our experiments. As we have pointed out repeatedly (3), (4), (5), (6), we have for a long time used the methods of auto-assay (by eye and blood-pressure reactions) solely for the purpose of corroborating results obtained by the rabbit segment method, which is more delicate and accurate.

The apparent discrepancy between the results of the pupil and segment estimations in our first observations was due, first of all, to the fact that the pupil observations cannot be made with the same accuracy as the segment assays, for the following reasons: *1.* Theoretically the adrenalin solution, which is injected to cause a dilatation of the pupil equal to that caused by the adrenal vein blood released from the pocket, must be introduced at the same rate as the blood from the pocket. This can only rarely be done. To make the conditions as similar as possible the adrenalin solution should probably be injected when the blood is free from epinephrin, the adrenal veins being clipped off. But even then the conditions are not precisely the same. For when the cava pocket is released the blood collected in it for the given time is followed up by adrenal blood containing epinephrin, which would tend to increase the dilatation. *2.* The pupil should be in precisely the same condition and should start with the same width in the observations which are to be compared. Now the pupil is apt to grow progressively wider and to constrict less completely after an epinephrin dilatation as the experiment goes on. This may soon render it impossible to continue the observations. *3.* The blood containing the epinephrin is necessarily greatly diluted before reaching the iris. In the case of the rabbit intestine segments the adrenal vein blood can be applied in whatever dilution is desired. As many observations can be made as are required for a satisfactory assay, provided enough blood has been secured, and observations can be repeated and their order varied at will. If one segment is not satis-

factory another can be substituted, but this cannot be done with the pupil.

Besides the inaccuracy inherent in the method, the average output deduced from pupil assays made in our first series (on 6 cats) (7) without comparison on rabbit segments, was probably rendered too high by the circumstance that the adrenalin was made up in Ringer's solution, in which it is more easily decomposed than in sodium chloride solution. Since then we have always employed the latter solution for making up the dilutions of adrenalin, whether they are to be injected into the circulating blood, or added to blood which is then to be placed in contact with the rabbit segments.

Five experiments in which the epinephrin output was estimated in the same cats both by pupil and by intestine segment assays can now be added to the two previously published. The results of the 7 experiments are given in table 2. All the animals were anesthetized with urethane except cat 814, which received ether. Details of the segment assays of the 5 new experiments are epitomized in table 1.

TABLE 2

CAT	EPINEPHRIN PER KILO PER MINUTE BY	
	Pupil	Segment
	mgm.	*mgm.*
85	0.0003	0.00024
86	0.0007	0.00014
814	0.0002	0.00029
815	0.0003	0.00053
816	0.0003	0.00037
817	0.00015	0.00018
821	0.0004	0.00076

Samples of the tracings obtained for the segment assay in cats 816, 817 and 821 are given in figures 2, 3 and 4.

In cat 816 numerous observations were made for about an hour in order to estimate the epinephrin output by the (denervated) eye reactions. The average was 0.0003 mgm. per kgm. per minute. Two samples of adrenal vein blood (referred to as the second and third specimens), besides a preliminary sample, were then collected, and at once assayed on a rabbit intestine segment. The flow was practically the same during collection of the two samples (1.85 grams and 1.77 grams per minute), and they gave practically identical reactions with the segment (fig. 2, *22* and *24*). In some observations the blood seemed somewhat stronger than 1:2,500,000 epinephrin (fig. 2, *20* to *24*) but in others slightly weaker, and in still other observations (fig. 2, *37* and *39*) about the same as 1:2,500,000. It was decidedly weaker than 1:1,875,000 (*22* to *26; 47* and *49*), stronger than 1:3,750,000 (*35* and *39; 43* and *47*),

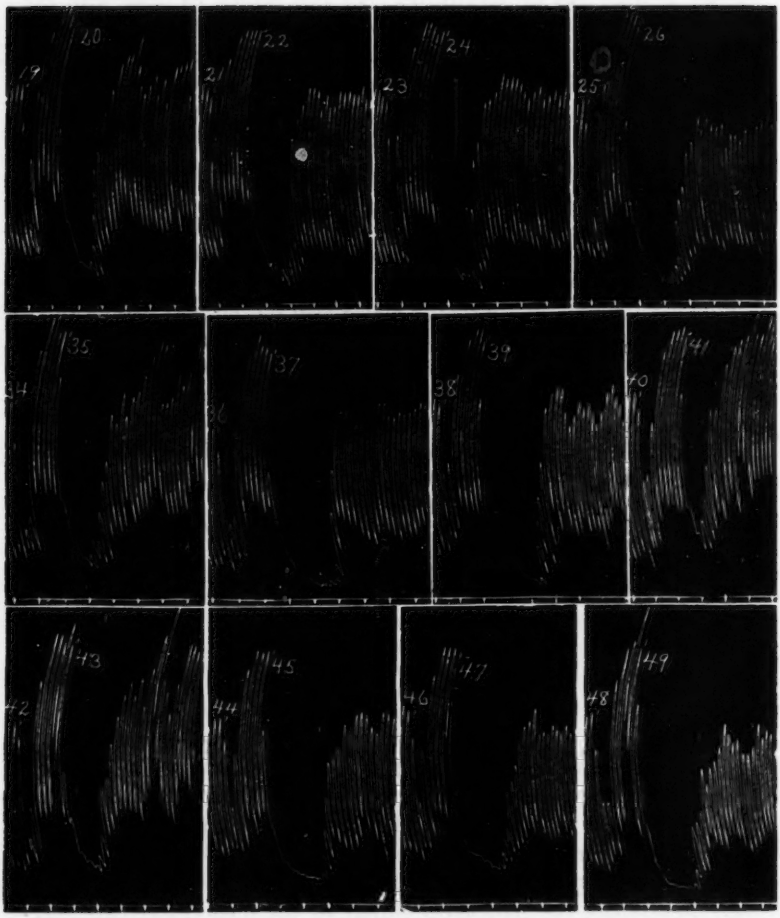

Fig. 2. Intestine tracings. Blood from cat 816. At *19*, *21*, *23*, *25*, *34*, *36*, *38*, *40*, *42*, *44*, *46* and *48* Ringer's solution was replaced by indifferent (arterial) blood, and this at *20* by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000; at *22* by the 2nd adrenal blood specimen; at *24* by the 3rd adrenal specimen; at *26*, *35* and *37* by indifferent blood to which adrenalin was added to make up concentrations of 1:1,875,000, 1:3,750,000 and 1:2,500,000 respectively at *39* by the 3rd adrenal specimen; at *41*, *43* and *45*, by indifferent blood to which was added adrenalin to make up concentrations of 1:5,000,000, 1:3,750,000 and 1:2,500,000 respectively; at *47* by the 3rd adrenal specimen; at *49* by indifferent blood to which was added adrenalin to make a concentration of 1:1,875,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). As in all the intestine tracings, the time is marked in half minutes. Reduced to one-half.

much stronger than 1:5,000,000 (*39*, *41*). Taking the blood at 1:2,500,000, we get (with a flow of 1.8 grams per minute) 0.00074 mgm. epinephrin per minute for the cat, or 0.00037 mgm. per kgm. per minute.

In cat 817 the left adrenal vein came off from the dorsal surface of the adrenal near the lower pole and coursed downwards and backwards, joining the renal vein not far from its opening into the cava. It was torn during the dissection, and the left adrenal had to be excised. The assays were therefore made with only the right adrenal discharging, and the estimated outputs have been doubled in the tables. A considerable number of observations were made on the pupil over a period of nearly two hours, after which it was too wide to be worked with. That the dilatation was in part being maintained by epinephrin coming at the ordinary rate from the right adrenal, was shown by the fact that when the cava pocket had been clipped off in order to collect blood for the segment assay the pupil diminished considerably, and the nictitating membrane came farther forward. The average output calculated for both adrenals from the pupil observations was about 0.00015 mgm. per kgm. per minute.

Blood collected from the adrenal after completion of the observations on the eye was found, with the rabbit intestine segment, to be much weaker than 1:1,250,000 adrenalin, and decidedly stronger than 1:3,750,000 (fig. 3, *24* and *28;* confirmed by several other tracings not reproduced). The blood was shown to be weaker than 1:1,875,000 adrenalin, and not much different from 1:2,500,000. In some of the observations the blood seemed possibly somewhat weaker than 1:2,500,000 (fig. 3, *20* and *22*) and in others possibly somewhat stronger, but it was proved that it could not have been far from this concentration of epinephrin. Taking the blood at 1:2,500,000, we get 0.0003 mgm. per minute as the epinephrin output for the right adrenal, or 0.00009 mgm. per kgm. per minute, corresponding to 0.00018 mgm. for both adrenals. Considering the smaller degree of exactness possible with the pupil assay, the agreement is close.

In cat 821 it was possible to use the eye for little more than half an hour for the purpose of estimating the epinephrin output, since at the end of that time both pupils had become wide. The average obtained was 0.0004 mgm. of epinephrin per kgm. of body weight per minute. The pupil assay could only be considered moderately satisfactory. It showed, however, that there was a good output.

The blood collected from the adrenal veins and assayed on the intestine segments was found to be much stronger than 1:4,000,000 adrenalin and than 1:2,600,000; also much stronger than 1:1,300,000 (fig. 4, *10* and *12*). As the inhibition produced by the blood was too great for accurate assay it was diluted with 2 volumes of indifferent blood. Thus

diluted, it was proved to be much weaker than 1:2,000,000, and probably slightly weaker than 1:2,600,000 (fig. 4, *22* to *26*), i.e., the blood was much weaker than 1:650,000 and probably slightly weaker than 1:870,000. The response of the segment having altered somewhat,

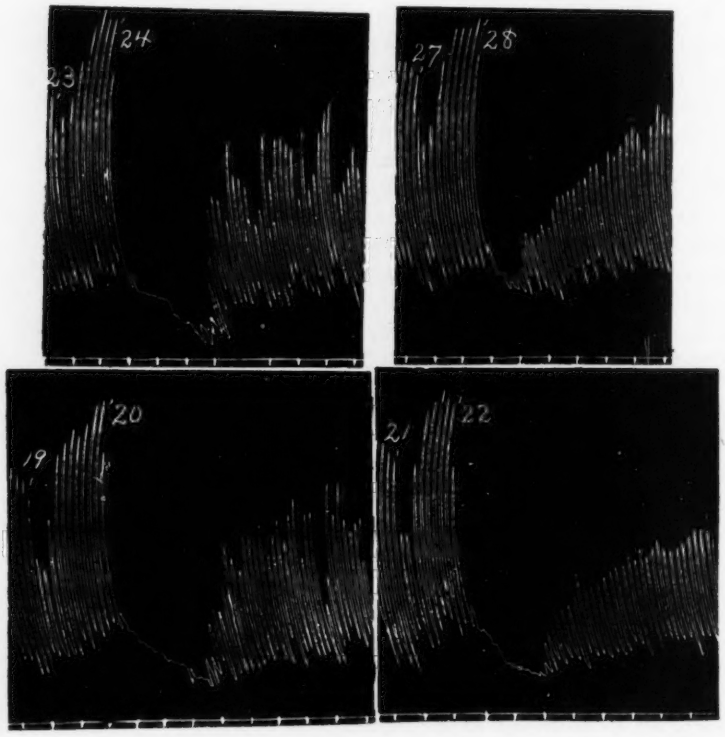

Fig. 3. Intestine tracings. Blood from cat 817. Segment atropinized. At *19*, *21*, *23* and *27* Ringer's solution was replaced by indifferent (arterial) blood, and this at *20* and *24* by the adrenal vein blood; at *22* and *28* by indifferent blood to which was added adrenalin to make up concentrations of 1:2,500,000 and 1:3,750,000 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fifths.

these observations were repeated, and the adrenal blood, diluted with two volumes of indifferent blood, was found to differ but little from indifferent blood made up with adrenalin to 1:2,600,000 (fig. 4, *30* and *32*), but to be much stronger than 1:4,000,000 (*34*), i.e., the adrenal blood was about the same as 1:870,000 and much stronger than 1:1,300,000. The adrenal blood, diluted with two volumes of indiffer-

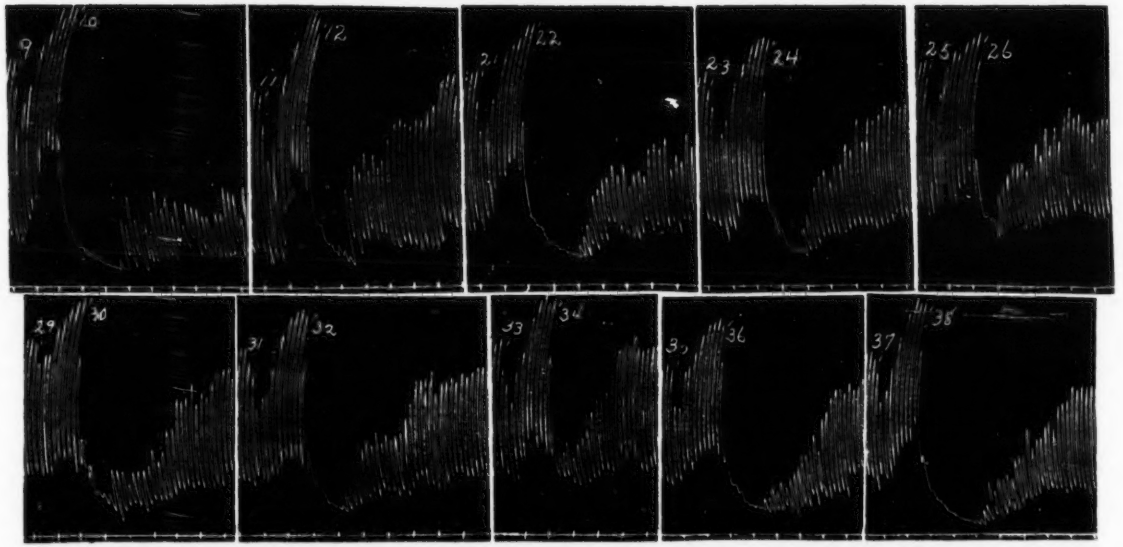

Fig. 4. Intestine tracings. Blood from cat 821. At *9*, *11*, *21*, *23*, *25*, *29*, *31*, *33*, *35* and *37* Ringer's solution was replaced by indifferent (arterial) blood, and this at *10* by the adrenal vein blood and at *12* by indifferent blood to which adrenalin had been added to give a concentration of 1:1,300,000. At *22*, *24*, *32*, *34* and *38* the indifferent blood was displaced by indifferent blood to which adrenalin had been added to make up concentrations of 1:2,000,000, 1:2,600,000, 1:2,600,000, 1:4,000,000 and 1:2,000,000 respectively. At *26*, *30* and *36* the indifferent blood was displaced by adrenal blood diluted with 2 volumes of indifferent blood. All the bloods were diluted with 3 volumes of Ringer's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

ent blood, was decidedly weaker than 1:2,000,000 (*36* and *38*), i.e., the adrenal blood was distinctly weaker than 1:660,000 adrenalin. Numerous additional observations confirmed the assay of the blood at about 1:900,000 epinephrin, corresponding to an output of 0.0015 mgm. per minute for the cat, or 0.00075 mgm. per kgm. of body weight.

The average output for the 7 cats, as estimated by the eye reactions, is 0.00034 mgm. per kgm. per minute, and as estimated by the intestine segments, 0.00036. No great weight should be attached to the close agreement, which is accidental. A very large number of experiments would be needed for an exact comparison, of which only those yielding good pupil assays ought to be used. The results of the 7 experiments, however, indicate that there is no deficiency in the output determined by the intestine segment method.

In two other cats (350 and 353) the output was determined by the eye reactions. In three additional cats (313, 314 and 318) and two dogs (278 and 351) an auto-assay by blood-pressure reactions was made. In these experiments no comparison was made by the intestine segment method. The results on these 7 animals are shown in table 3. Each output is the average of a number of observations. The average output per kilogram per minute for the 7 animals, as estimated by auto-assay (pupil and blood pressure), is 0.00023 mgm.

TABLE 3

NUMBER OF ANIMAL	SEX	BODY WEIGHT	WEIGHT OF ADRENALS	EPINEPHRIN OUTPUT		ANESTHETIC
				Per minute	Per kgm. per minute	
		kgm.	*gm.*	*mgm.*	*mgm.*	
350	M	1.82	0.410	0.00035	0.0002	U
353	M	3.13	0.705	0.00037	0.00012	U
313	F	3.0	0.319	0.0009	0.0003	E
314	F	2.12	0.587	0.0004	0.0002	U
318	M	3.3	0.367	0.0019	0.00057	U
278	F	5.6		0.0006	0.0001	M, E
351		6.3		0.0009	0.00015	M, E

The last 2 animals in the table are dogs, the rest cats.

The average for the auto-assays of the 14 animals in tables 2 and 3 is 0.00028 mgm. per kgm. per minute.

In one cat (no. 91), after extirpation of one adrenal and denervation of the other, the residual output of epinephrin, two months thereafter, was estimated at 0.00007 mgm. per kgm. per minute by the pupil reaction and 0.00005 mgm. by the rabbit segments, showing no significant difference, considering the small output of epinephrin.

A more accurate way of determining whether any sensible loss of epinephrin is incurred in defibrinating the blood drawn for assay with the rabbit intestine segments is to add the blood directly from a cannula in an artery to known amounts of adrenalin, before defibrinating it, and to add other samples of the same blood, after defibrination, to adrenalin, so as to make up the same theoretical concentrations. The samples are placed together on ice and then assayed. If loss of adrenalin has occurred during defibrination this will be apparent when the samples are tested on the intestine segment. The question whether any appreciable loss is entailed when the samples of defibrinated adrenal vein blood are allowed to stand on ice till all have been collected before the assay is begun, can be tested by making up a given theoretical concentration of adrenalin in the freshly defibrinated carotid blood and seeing whether the assay yields the same value at varying intervals from the time the blood was drawn, and whether this value tends to diverge from the theoretical value when the blood stands for the few hours over which the assay of several specimens of adrenal vein blood may extend. Six experiments of this kind were made. Generally the blood was obtained from the carotid of rabbits, and the intestine segments from the same animal. But in one instance the blood was drawn from a cat and assayed, of course on a rabbit intestine segment. The animals were anesthetized with ether during insertion of the cannula, and as the blood was then immediately obtained, the total period of anesthesia was short. Defibrinated blood from the same animal was used as the "indifferent blood." The adrenalin was made up in a small volume of sodium chloride solution.

It may be said at once that the results of these experiments were quite consistent, and demonstrated clearly that neither during defibrination nor during standing on ice for a few hours was there any loss of adrenalin detectable by the segments. In one instance a sample of the defibrinated blood to which adrenalin had been added was allowed to stand overnight in the ice chest, and then tested. Even in this case an excellent inhibition of the segment was caused, and it was shown that the loss of adrenalin was not great. In another instance, where the defibrinated blood after the addition of adrenalin was left 4 days in the ice chest the concentration of adrenalin was of course much reduced (to about one-fifth of its original value).

In cat 812 four specimens were obtained from the carotid, as follows:

First specimen run into adrenalin to make up a concentration of 1:2,500,000. The blood and adrenalin solutions were rapidly mixed and the blood then defibrinated.

Second specimen similar to the first, except that the concentration was 1:1,250,000.

Third specimen: adrenalin was added to defibrinated blood to make up a concentration of 1:2,500,000.

Fourth specimen similar to the third, but the concentration was 1:1,250,000.

The blood contained a large amount of serum (80 per cent). Assuming that all the adrenalin remained in the serum, the concentration in the serum would be 1:2,000,000 in the first and third specimens and

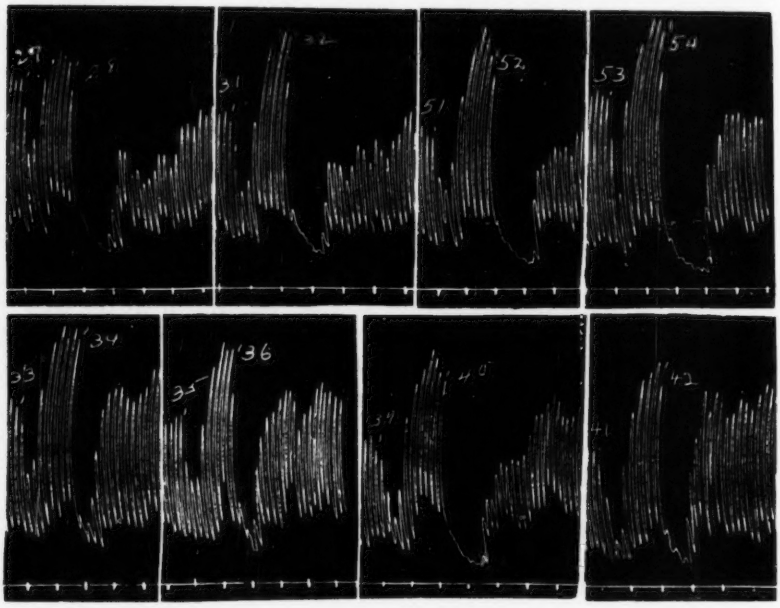

Fig. 5. Intestine tracings. Blood from rabbit 812. At *27*, *31*, *33*, *35*, *39*, *41*, *51* and *53* Ringer's solution was displaced by indifferent (arterial) blood and this at *32* and *52* by the 4th specimen; at *34* and *36* by the 1st and 3rd specimens respectively. At *28*, *40* and *54*, the indifferent blood was replaced by indifferent blood to which adrenalin had been (freshly) added to make up a concentration of 1:1,250,000. At *42* the indifferent blood was displaced by indifferent blood to which adrenalin had been (freshly) added to make up a concentration of 1:3,125,000. All the bloods were diluted with 3 volumes Ringer before application to the segment (the adrenalin blood after adding the adrenalin). Reduced to three-fifths.

1:1,000,000 in the second and fourth. The specimens were placed upon ice as soon as drawn off, and 2 hours later the assay was begun. It was completed in $3\frac{3}{4}$ hours.

Some samples of the tracings used in the assay are reproduced in figure 5. The first specimen gave practically the same reaction as the third (fig. 5, *34* and *36;* confirmed by other observations not reproduced). There was no essential difference in the reaction given by either the first or the third specimen and that yielded by a dilution of adrenalin, 1:2,500,000, freshly made up in the defibrinated blood. The same was found to be the case after the first and third specimens had stood 5 hours on ice. They were both decidedly stronger than 1:3,125,000 and much weaker than 1:1,250,000 adrenalin freshly made up in the defibrinated blood (fig. 5, *42* and *40*). The fourth specimen after standing 2 hours on ice gave practically the same reaction as 1:1,250,000 adrenalin freshly made up in the defibrinated blood (fig. 5, *28* and *32*). After the fourth specimen had stood 5 hours on ice the inhibition caused by it was not less than that caused by defibrinated blood freshly made up to a concentration of 1:1,250,000 adrenalin (fig. 5, *52* and *54*). A number of additional observations, not reproduced, confirmed the conclusion that no sensible loss of added adrenalin had occurred either in defibrination of the blood, or while the blood stood on ice during the assay.

In rabbit 813 four specimens of carotid blood were prepared in the same way as in cat 812. The first and second specimens were mixed with adrenalin solution to make up concentrations of 1:950,000 and 1:1,900,000 respectively and then defibrinated. The third and fourth specimens were made up by adding adrenalin to already defibrinated blood to concentrations of 1:950,000 and 1:1,900,000 respectively. The blood contained 75.3 per cent of serum. The assay of the blood specimens on an intestine segment from the same rabbit was begun 50 minutes after the blood was drawn. Samples of the tracings are reproduced in figure 6.

The first and third specimens gave practically the same reaction, and so did the second and fourth. As shown in figure 6 (*22* and *24*), the second specimen gave the same reaction as 1:1,900,000 adrenalin freshly made up in the defibrinated blood. The reaction given by the fourth specimen was similar (*16*), and in other observations (not reproduced) practically identical. The reaction given by a freshly prepared dilution of adrenalin 1:950,000 in the defibrinated blood (*20*) was of course decidedly stronger than that given by the second and fourth specimens, and about the same as that yielded by the first and third specimens (tracings not reproduced). It was shown that the first and third specimens were distinctly weaker than 1:630,000 and distinctly stronger than 1:1,270,000 adrenalin freshly made up in the defibrinated blood. Several other sets of observations confirmed the practical identity of the reactions yielded by the first and third specimens and freshly made up adrenalin, 1:950,000, and of the reactions yielded by the second and

fourth specimens and freshly made up adrenalin 1:1,900,000. The assay was finished in 4 hours.

The specimens were then placed in the refrigerator and reassayed 4 days (100 hours) later, of course on a segment from another rabbit. The third and fourth specimens gave about the same reaction (fig. 6,

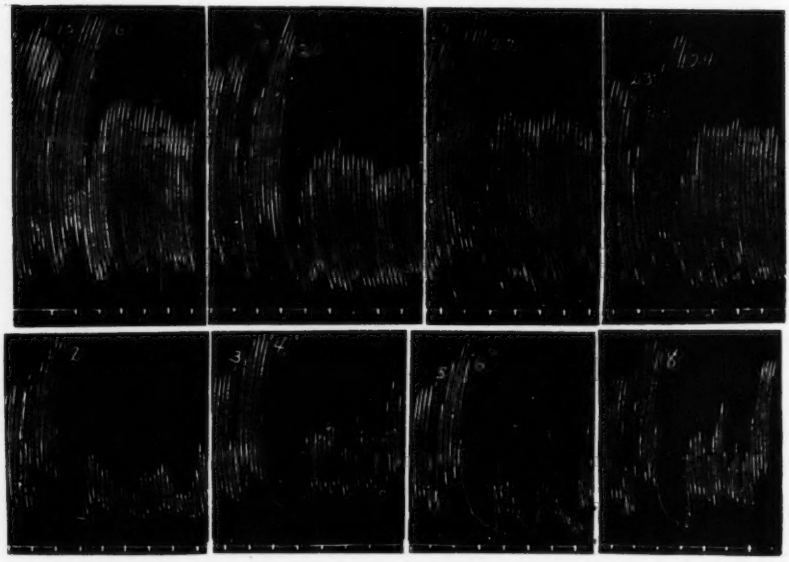

Fig. 6. Intestine tracings. Blood from rabbit 813. The upper row of tracings is from the assay of the blood specimens shortly after being made up. The lower row of tracings is from the assay on an intestine segment from another rabbit of the same blood specimens after standing 100 hours longer on ice. At *15*, *19*, *21* and *23* Ringer's solution was replaced by indifferent (arterial) blood and this at *16* and *24* by the 4th and 2nd specimens respectively, and at *20* and *22* by indifferent blood to which adrenalin had been (freshly) added to make up concentrations of 1:950,000 and 1:1,900,000 respectively. At *1*, *3*, *5* and *7* Ringer's solution was replaced by the indifferent blood and this at *2*, *4*, *6* and *8* by the 1st, 3rd, 2nd and 4th blood specimens respectively (after standing 100 hours on ice). All the bloods were diluted with 3 volumes Ringer before application to the segments (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

observations *4* and *8*), the third being probably somewhat the stronger of the two. The first and second specimens were not very different in the amount of inhibition produced, the first being probably somewhat the stronger. Both were stronger than the third and fourth specimens (fig. 6, observations *2* and *6*). In other observations it was seen that

the first specimen was stronger than the second, third and fourth. It is obvious that no great uniformity could be expected in the rate of loss in the various specimens, since they were shaken up, some more, some less, and were not even kept in tubes of the same cross section. The only object of the comparison was to show that even after long standing of these relatively weak solutions of adrenalin in blood, concentrations easily detectable by the segments were still present. It may indeed be pointed out that the reactions obtained were actually much greater than with the fresh specimens. This, of course, depends merely upon the fact that the segment used for the second assay was more sensitive than that employed for the first. The first specimen was in one set of observations somewhat weaker than 1:6,250,000 adrenalin, but in another set there was not much difference. The first specimen had therefore lost more than five-sixths of its adrenalin. The second specimen was shown by a number of observations to be not greatly weaker than 1:6,250,000. It had therefore lost not much more than two-thirds of its adrenalin, being originally only half as strong as the first specimen. The fourth specimen was decidedly weaker than 1:7,500,000 and stronger than 1:12,500,000. It was not thought worth while to assay it more exactly, but it had probably lost at least three-fourths of its adrenalin.

In another experiment (rabbit 852) a specimen of defibrinated blood made up with adrenalin to a concentration of 1:1,500,000 was allowed to stand for 24 hours in the refrigerator. It gave a very large reaction (fig. 7, *46*), much larger than that given by blood freshly made up with adrenalin to a concentration of 1:2,500,000. It was not thought worth while to assay the specimen more exactly, but it could not have lost more than one-fourth of its adrenalin, if so much. A specimen of blood collected from the carotid, made up with adrenalin to 1:1,500,000 and then defibrinated and assayed forthwith, gave similar reactions to those yielded by a specimen with the same concentration made up in the defibrinated blood.

In rabbit 854 the first specimen was carotid blood made up with adrenalin to a concentration of 1:1,250,000 and then defibrinated, and the second, defibrinated blood made up to the same concentration. The blood specimens were placed on ice in the usual way and at once assayed. Some samples of the tracings are reproduced in figure 7. The first and second specimens gave about the same reactions, which were also similar to those yielded by defibrinated blood freshly made up with adrenalin to a concentration of 1:1,250,000. Both specimens were shown to be very much stronger than 1:2,500,000 adrenalin. The segment was now atropinized and the assay repeated, the blood specimens being henceforth diluted with 3 volumes of 0.9 per cent sodium chloride solution and

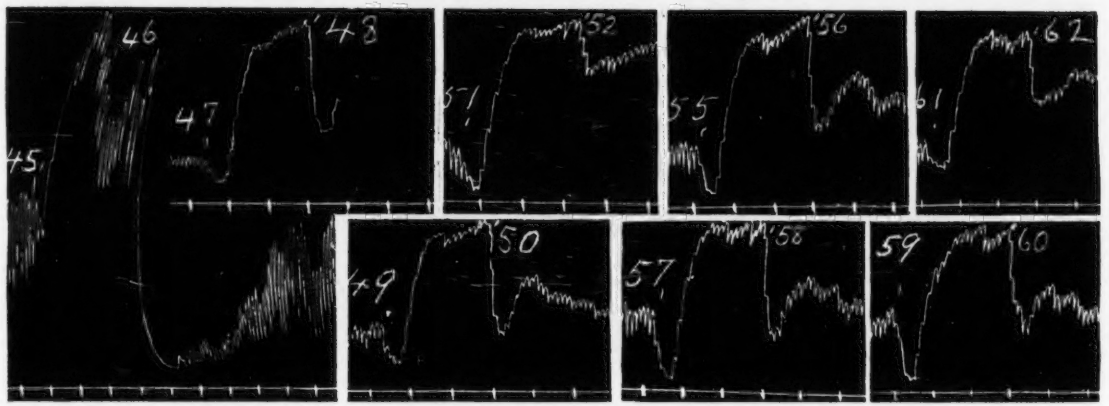

Fig. 7. Intestine tracings. At *45* Ringer's solution was replaced by indifferent (arterial) blood from a rabbit (852) and this at *46* by a specimen of the defibrinated blood made up with adrenalin to a concentration of 1:1,500,000 and allowed to stand 24 hours on ice. The bloods were diluted with 3 volumes Ringer before application to the segment. All the other tracings in the figure are from the assay of blood specimens from another rabbit (854) on one of its own intestine segments. Tracing 45 and 46 was from a segment of another rabbit. At *47*, *49*, *51*, *55*, *57*, *59*, and *61* Ringer was replaced by indifferent (arterial) blood from rabbit 854, and this at *48* and *50* by the 1st and 2nd blood specimens (from rabbit 854) respectively; at *52*, *56* and *62* by indifferent blood (freshly) made up with adrenalin to concentrations of 1:2,500,000, 1:1,250,000 and 1:1,875,000 respectively; at *58* and *60* by the 1st and 2nd specimens respectively. All the bloods were diluted with 3 volumes of 0.9 per cent NaCl solution and 2 volumes Ringer's solution before application to the segment, which had been atropinized before observation *47*. Reduced to four-fifths.

2 volumes of Ringer's solution, instead of 3 volumes of Ringer's solution[2] alone, as usually employed. This was done in order to reduce the increase of tone caused by the indifferent blood, which was considerable. The results of the assay were precisely the same as before atropinization of the segment, although the appearance of the curves was different. The first and second specimens gave about the same reaction with the segment (fig. 7, *48* and *50*). They were much stronger than 1:2,500,000 adrenalin (52) about the same as 1:1,250,000 (*56*, *58* and 60), and decidedly stronger than 1:1,875,000 (*62*).

The last of these experiments to be cited is that on rabbit 855. The first and second specimens were made up, as in the experiment on rabbit 854, to a concentration of 1:1,250,000 adrenalin, the first before, and the second after defibrination of the blood. Some samples of the tracings used in the assay are reproduced in figure 8. The first and second specimens caused about the same effect on the rabbit segment. This effect was also much the same as that produced by defibrinated blood freshly made up with adrenalin to a concentration of 1:1,250,000. The segment was now atropinized and the observations repeated with a similar result (fig. 8, observations *12*, *14* and *16*). This was confirmed by another set of observations. Atropine was again applied to the segment with an improvement of the curves. The first blood specimen now gave a reaction, if anything, slightly stronger than that produced by 1:1,250,000 adrenalin freshly made up in the defibrinated blood; and in the next observation (*28*) the first specimen looked, if anything, a little weaker than 1:1,250,000, but decidedly stronger than 1:1,875,000 adrenalin (*30*). In other observations, not reproduced, it was shown that the first specimen was not weaker than 1:1,250,000 and much stronger than 1:1,875,000. The second specimen caused reactions very similar to those given by the first specimen, sometimes appearing slightly stronger (*34*, *36*) sometimes slightly weaker, but the reaction given by both was not less than that caused by 1:1,250,000 adrenalin freshly made up in the defibrinated blood (*38*). About 4 hours after the blood was obtained the second specimen seemed to be slightly weaker than 1:1,250,000 adrenalin, but in another observation it seemed quite as strong, although now the first specimen seemed a little weaker than the second. It must be remembered that every time blood was taken from a tube to be applied to the segment the blood was shaken up, so that it would not be astonishing if a slight loss, not necessarily the same in all the specimens, should occur occasionally in the course of a

[2] The Ringer's solution employed by us is made up as follows: $NaHCO_3$ 1 gram dissolved in distilled water and added to NaCl 90 grams, KCl 4.2 grams, $CaCl_2$ 2.4 grams, dissolved in distilled water. Made up to 10 liters.

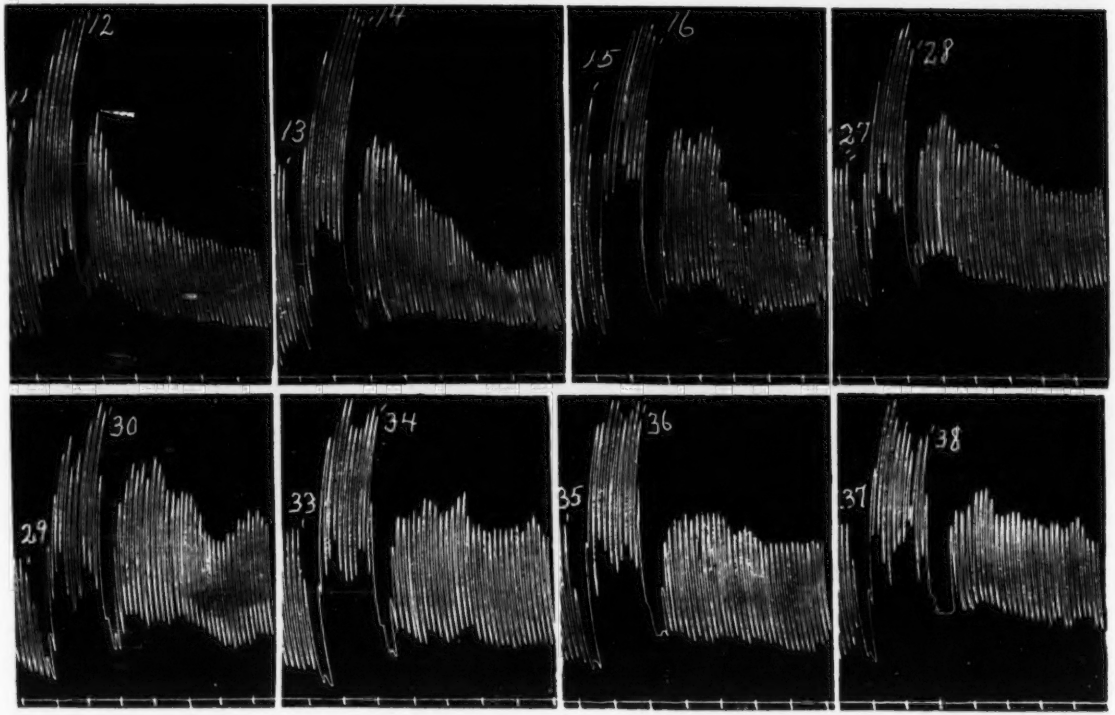

Fig. 8. Intestine tracings. Blood from rabbit 855. At *11*, *13*, *15*, *27*, *29*, *33*, *35* and *37*. Ringer was replaced by indifferent (arterial) blood and this at *12*, *28* and *34* by the 1st blood specimen; at *14* and *36* by the 2nd specimen; at *16* and *38* by indifferent blood made up (freshly) with adrenalin to a concentration of 1:1,250,000; at *30* by indifferent blood made up (freshly) with adrenalin to a concentration of 1:1,875,000. All the bloods were diluted with 3 volumes Ringer before application to the segment, which had been atropinized before *11* and again between *16* and *27*. Reduced to two-thirds.

long assay. It was shown, however, conclusively that this, if it occurs, is negligible, cannot usually be demonstrated by the segment method, and therefore cannot sensibly interfere with the assay.

In view of the results obtained for the output of epinephrin in normal animals we are unable to take seriously the statement of Hartman, McCordock and Loder (8) that in surgical operations the discharge of epinephrin is greatly augmented, every cut through skin and muscle, opening of the abdominal cavity, even careful handling of the intestines, etc., producing greater and greater increases in the output. Anesthetizing with ether, they consider, markedly increases the output. We did all of these things in obtaining adrenal blood for assay, and therefore if there was any basis whatever for the assertion of these writers we ought to have obtained quite large quantities of epinephrin. An output of 0.0001 mgm. of adrenalin per kgm. of body weight per minute and even less was sometimes found. This would not probably suffice to maintain a concentration in the arterial blood of more than 1:2,000,000,000, if no epinephrin is lost in the lungs. But this concentration of 1:2 billions, according to Hartman and his associates, must represent not only any concentration originally present in the "intact, non-anesthetized, quiet animal" but a great increase due to the operation and the anesthetic. Each successive step in this increase they claim to be able to demonstrate, so much for each cut in skin or muscle, so much for touching the intestines, so much for the ether, and so on. If our operative procedure only increased the output 10 times, then their claim amounts to this, that starting with a concentration of say, 1:20 billions in the arterial blood they can demonstrate successive increases by definite stages up to 1:2 billions. According to them this must represent a great outpouring of epinephrin, far beyond anything which can be present normally, giving maximal reactions with the test object they employ (the iris denervated by removal of the ciliary ganglion and the superior cervical ganglion) and "indicating an emergency function of the adrenals." Of course they furnish no quantitative data whatever, not one estimation of the rate of output or of the concentration either in the adrenal vein blood or the arterial blood, nothing but pictures showing a greatly dilated pupil under the conditions in which we find from 0.0001 mgm. to 0.0003 mgm. of epinephrin per kgm. of body weight per minute actually coming off from the adrenals. A progressive dilatation of the pupil as an experiment goes on is a very common thing indeed. This has been abundantly proved in our own work to be no indication of increasing epinephrin output. On the contrary, samples of adrenal vein blood collected at intervals throughout the experiment have shown the same output, quite without relation to the size of the pupil. In animals whose epinephrin output has been suppressed similar changes occur in the pupil during an experiment and the pupil may be and long remain maximally dilated after death. Yet, as we have pointed out (9), it can often be proved that the epinephrin coming off at the ordinary rate is exerting an action on the iris, since a pupil caused to dilate by injection of adrenalin returns to its original size more rapidly, and a pupil which has grown progressively wider sometimes becomes narrower when the adrenal veins are clipped. This is under conditions in which the ordinary rate of epinephrin output has been shown to be present. This is the answer to Kellaway's (21) suggestion that "the adrenaline normally present in the blood is not near the threshold necessary to cause the paradox." If it is near or actually at the threshold and holding the pupil dilated to some extent, it seems very probable that other influences which cause dilatation of the pupil, such as asphyxia, ether, and the

chemical or other effects of fatigue may be favored when the adrenals are discharging epinephrin, and may therefore produce a greater effect than in the absence of epinephrin, although they do not cause any increase in the epinephrin output. So that when Hartman and his associates photograph a pupil which has become narrower after removal of the adrenals, they do not show that the output of epinephrin was increased, but merely, at most, that the adrenals were giving off epinephrin, which we, at any rate, are willing to admit.

It is a sheer absurdity to say that they can tell that this output has been greatly augmented, and in successive stages, by the operative procedure. They make the point that ether does not prevent a marked increase in the rate of epinephrin discharge, caused by sensory stimulation, as shown by dilatation of the pupil. Now we found (10) that with ether, urethane and brain pressure without chemical anesthesia, stimulation of sensory nerves caused no detectable increase in the epinephrin output, as estimated by assaying the adrenal vein blood on rabbit intestine (and uterus) segments. If stimulation of sensory nerves causes a greatly increased output of epinephrin in etherized animals, how is it possible to miss it in the adrenal vein blood where it must first appear? The same question may be asked in regard to the asphyxial increase, which they say is the greatest of all. The epinephrin must first get into the adrenal vein blood, and it is this blood which we collect. Our own explanation of the discrepancy is that we are assaying the epinephrin, whereas they are simply interpreting erroneously changes in the pupil. In any case if the reader cares to compare those dilated pupils with the outputs given in our tables and in figure 1, he will be able to judge for himself how much the reaction employed by these writers can be relied upon to determine whether the output is markedly augmented or not. So that when they say "We are able to draw but one conclusion, the 'cava pocket' method does not permit an estimation of the output of epinephrin which prevails in a quiet intact animal", we may point out that they can safely say the same about the method employed by themselves. There is this great difference, however, between the results of the two methods, even as regards the single question of the output in intact animals, that we give data by which it may be seen whether and how the measured outputs are affected by varying the surgical procedures, the anesthesia, the kind of animal, etc., and whether the outputs are above or below the thresholds necessary for eliciting given physiological reactions. They further permit the study of the question whether and in what degree the outputs are increased or decreased under the influence of added factors such as drugs, and so on.

When the reader looks at one of the pictures of the eye reproduced by Hartman and his colleagues, and is duly impressed by the widely dilated pupil at the end of a series of surgical procedures and by the regular machine-like increase in successive steps as the epinephrin comes pouring out under the influence of the ether, the skin incision, the putting on of the skin clamps, the incision through the abdominal wall, and the pushing aside of the intestine, it may occur to him to ask how great an output is actually present at the climax of these successive outbursts. The observers who photographed the eye will not tell him, because they do not know. They leave him to gather that there must have been quite a lot of epinephrin around as the result of the "emergency function" of the adrenals. If the curious reader now turns to our tables or to figure 1 he will see how much epinephrin was actually given off under similar operative procedures in a large number of animals, and he will perhaps be disappointed to find that all these outbursts have together produced the modest outputs there displayed. He may

further be impressed with the following facts: that the output in one and the same animal is not increased as an experiment goes on for hours, but remains practically unchanged; that the nature and depth of the anesthesia do not seem to affect the output materially; that it does not seem to make any material difference whether the abdomen is opened or the adrenal vein blood obtained by the lumbar route; that the average output in sufficiently large groups of animals, even of different species, is singularly constant; that neither the magnitude nor the distribution about the mean of the separate determinations is such as to suggest that the experimental conditions modify them essentially. The etherized animals, indeed, actually have a slightly smaller average output than those under urethane, although Cannon (11) has stated that urethane exerts no demonstrable influence upon the output. Putting all these things together, the reader may perhaps be inclined to conclude that after all it is likely that what we have been measuring is a quantity not materially influenced by the procedures adopted by us to obtain the adrenal vein blood, where alone at present the epinephrin concentration can be satisfactorily assayed. In any case, we hope that he will agree with us that it is better to measure than to guess.

Not only do the changes in the pupil not mean what Hartman, McCordock and Loder think they mean, but even a glance at some of their data shows that they have used them in a quite illogical way. For example, they state that "the barking dog" does not cause any dilatation of the denervated pupil or causes a slight dilatation both before and after the removal of the adrenals. Instead of concluding, as they ought, either that their method is wrong, since Cannon (12) found that this form of psychical stimulation caused such a greatly increased discharge of epinephrin in cats that even the mixed venous blood became quite distinctly charged with it, or that psychical excitation does not increase the output, they conclude that Cannon's observations on the physiological potency of the barking dog are all right *and* that their method of demonstrating increases in epinephrin output is certain and sensitive. Our own opinion is that both methods are quite inadequate; and that the exploits of the barking dog in frightening epinephrin out of the adrenals of a cat being a myth, the wisest and kindest thing his friends can do is to bury and not to praise him.

It may be noted that in the paper under discussion it was only procedures causing direct physical effects upon the animals which produced a well-marked dilatation of the pupil. Now there are many changes which asphyxia, etherization, etc., may and do produce besides affecting the epinephrin output, and these would have to be excluded far more thoroughly than was done before the results could have any value. That removal of the adrenals in an acute experiment might affect the dilatation when the pupil test was instituted in 2 to 5 hours thereafter, without indicating that the dilatation produced before the adrenalectomy was due to increased epinephrin output, has been clearly shown in regard to other reactions (13), (14). Nevertheless in a notable proportion of the cats asphyxia gave some dilatation after removal of the adrenals, the pupillary breadth being more than trebled in one case, doubled in another, etc. Since intervals of as long as 3 days occurred between the two tests there is no guarantee that the sensitivity of the pupil remained the same.

It is stated that "if infection should occur the pupil remains widely dilated." Anderson (15), who observed animals for a long time after removal of the superior cervical ganglion and the ciliary ganglion, the latter by Jegorow's (16) method, was inclined to attribute a so-called paradoxical constriction of the pupil to absorption of substances from a recent wound.

We forbear to discuss the paper further. The assertion that the epinephrin output is much increased by cold is no better founded than the others. Even if true, we do not think any of these statements could be proved to be true by this method. So far as this goes, Rapport's (17) statement that in shock the output is increased, because in 6 out of 9 anesthetized animals he sooner or later saw some acceleration of the (denervated) heart, is in the same position. If one is to interpret every change in the rate of the denervated heart over a long experiment, or every change in the denervated pupil, as indicating changes in the epinephrin output, many startling "results" will undoubtedly be written into the literature, but shall we get any farther forward with the detection and measurement of such changes in the output as do occur?

Hartman, Waite and Powell (18) have published another paper, purporting to show that the physiologically "secreted epinephrin plays a very important rôle in increasing muscular work and delaying the onset of fatigue". This conclusion is based upon observations made on normal cats and on cats deprived of one adrenal and with the other denervated, when made to run in a revolving cage. Shortly before this we published (19) experiments made in the same way, but with an opposite result. Comparison of the conclusions of Hartman and his fellow workers with such protocols as they have given show that no exact deductions can be made as to the relative endurance of the normal and operated animals. And certainly anything like an exact comparison would be difficult. Yet if the epinephrin plays "a very important rôle," it should be easy to detect a difference. We do not believe this conclusion has any foundation whatever. Our operated cats differed in one respect from those used by Hartman and his collaborators. Five of them had one adrenal and from $\frac{1}{2}$ to $\frac{3}{5}$ of the other removed, so that there was a marked anatomical deficiency of adrenal tissue, both cortex and medulla. This operation was chosen because we considered that if the adrenal influences muscular fatigue at all, it is more likely to be through the cortex than through the epinephrin output. In three of these animals the remaining fragment was ultimately denervated. In a sixth animal one adrenal remained, but denervated. Later on $\frac{3}{4}$ of this adrenal was excised. We found it impossible to establish any clear difference in susceptibility to fatigue or rapidity of recovery from it between the operated and the normal cats. Some of the animals had convulsions, but this is also true of normal animals; and instances are also given in our paper of cats with one adrenal denervated and the other normally innervated, and therefore discharging epinephrin, which went into convulsions. We note in the paper under discussion that some of the operated animals were worked very hard, being fatigued daily, in one instance 6 days in one week, with several repetitions on some of the days. It is stated that observations were made before the operation but not to the point of great fatigue. It is difficult to see how a comparison can be properly made in this way.

Hartman cites observations made by Batelli and Boetta (20) as showing that the epinephrin store of the adrenals is greatly reduced by severe muscular exercise in dogs. Batelli used a method which would hardly be employed now (his ferric chloride method). But a more serious objection to his work is that he did not compare a "protected" adrenal with its still innervated fellow, according to Elliott's excellent plan, but simply compared the average store per kilo and the variations in 7 fatigued dogs with the average and the range he had found at various times in normal dogs. We (19) made estimations by the colorimetric method of Folin, Cannon and Denis on 5 cats, with one adrenal denervated according to Elliott's method, and found that it was only when muscular exertion

was carried to the possible limit that a definite depletion of the still innervated gland, as compared with its denervated fellow, was produced. Even very considerable fatigue may be associated with complete equality in the stores of the two glands. When a moderate depletion is found after convulsions it must be remembered that this may be associated with asphyxia. In any case, it would be difficult to associate directly with the muscular exertion the moderate depletion observed, and impossible to say whether it was due to an increased epinephrin output.

Our observations on the behavior of a very large number of animals in which the epinephrin output had been interfered with, kept in the laboratory under similar conditions to the normal animals and indistinguishable from them, are of much greater value, we believe, than these so-called fatigue experiments. Hartman, Waite and Powell say that "it would be interesting to know whether such animals could meet conditions of stress as well as normal animals. So far as we know the animals of Stewart and Rogoff were permitted to lead a quiet life as most laboratory animals do." Our operated cats, when recovered, were allowed to lead the same life as the normal animals, in summer in the open air on the roof. They were subjected to whatever "stresses" occur normally in a group of ordinary cats of both sexes, including, we suppose, a certain amount of fighting. They were observed to be equal to all the "emergencies" of ordinary cat life, including pregnancy.

No so-called "temperamental" changes were observed which could with any probability be attributed to the loss of epinephrin. This is pointed out because some writers have laid stress on such changes. In a recent paper (21) it is stated that "the most notable change (after removal of one adrenal in cats and partial destruction of the other by radium emanation) is in the temperament of the animals, which became quieter and more subdued. A subcutaneous injection of 0.5 mgm. of adrenaline generally restores the animal for a time to its normal preoperative condition. One animal which was rather wild before operative interference with its adrenals became thoroughly tame, but reverted to its wild state after the injection of adrenaline." It is striking that a small dose of adrenalin administered subcutaneously should cause the veneer of civilization, artificially acquired by interference with the epinephrin output, to peel off so suddenly. But this scratching of a Russian and finding a Tartar is so common an experience in injecting other things than adrenalin into our operated cats that we cannot share the view that adrenalin has any special influence.

Rabbits surviving double adrenalectomy behave precisely like ordinary rabbits, although no epinephrin from the adrenals is present. We mated two pairs of rabbits after complete adrenalectomy of both males and females. Pregnancy and parturition were normal, one mother giving birth to 5 and the other to 7 young. Rats surviving double adrenalectomy behave normally, and as demonstrated long ago by Boinet (22), show no essential difference from normal rats as regards resistance to fatigue.

Hartman, Waite and Powell say they have tested in one instance the effect of pregnancy in a cat with reduced epinephrin output. Seventy-six days after removal of the right adrenal and the left semilunar ganglion, "the adrenal and lumbar veins to the remaining adrenal were tied. The cat became pregnant, six healthy kittens being born 182 days after the primary operation," i.e., 106 days after ligation of the veins of the remaining (left) adrenal. There is no discussion of this observation, no mention of autopsy findings and no conclusion as to the bearing of the observation on the relation of the adrenal to pregnancy and parturition.

In some of their animals the superior cervical ganglion had been removed, and the changes in the pupil during fatigue were studied. These writers believe that the output of epinephrin in fatigue can be tested by the changes in the pupil of the denervated eye. "A cat which shows a good dilatation of the denervated pupil accompanying work in the treadmill is able to do more work than it can when such dilatation is absent. Spurts of work are accompanied by greater increases in the dilatation". In several other places they insist that the cause of the dilatation is the cause of the increased power of work. This seems to us to be putting the cart before the horse. The true sequence we believe is that when the cat works harder the pupil becomes more dilated, not that when the pupil becomes more dilated the cat is able to work harder. The dilatation of the pupil of the denervated eye can be associated with other things than epinephrin: ether, morphine, asphyxia, sometimes emotional disturbance will cause it to dilate and even to become wider than the pupil of the normal eye in animals whose epinephrin output has been greatly interfered with or abolished. There are other ways in which fatigue might occasion widening of the pupil than by increasing the output of epinephrin. Until it is shown by a proper method that fatigue does increase the output, much of the reasoning in this paper is based on a pure assumption.

When it is found that in some of the experiments animals which have been subjected to the adrenal operation also show dilatation of the denervated pupil, the assumption that this is due to an increased outpouring of epinephrin is saved by another assumption, that regeneration of the epinephrin-secretory fibers has occurred. It has never been shown that this takes place, and therefore, of course, it has never been shown how long an interval may be required for it to take place. The assertion is made that in one case "168 days after the operation the fatigue test again elicited dilatation of the denervated pupil. . . . . The adrenal was again exposed and found to be connected with nerve fibers from the splanchnic nerve" . . "Nerves had regenerated from the splanchnic to the adrenal." In another cat "from the time that the animal was first tested as to the pupil reaction (53 days after removal of one adrenal and cutting the nerves to the other) the denervated pupil always showed some response, which we thought might be explained by regeneration of the nerve fibers. At autopsy (61 days after adrenal operation) this appeared to be the case because nerve fibers again connected the semilunar ganglion with the adrenal". . . "In another cat which possessed but one adrenal and that denervated, a good eye reaction was obtained accompanying work. At autopsy, nerve fibers were found connecting the splanchnic with the adrenal." We do not see how it is possible by mere inspection to determine that nerve fibers have regenerated.

Their observations, they say, seem to indicate that this nervous control (of the adrenals) bears an important relation to the muscular efficiency of the animal. How can this be the case if we have seen some hundreds of animals deprived of this nervous control, rabbits permanently, monkeys for as much as 9½ months, dogs and cats for months, living not only in good health but as vigorous in every way as normal animals and not to be distinguished from them? They proceed, "Tests of this must be made before the nerve fibers have time to regenerate. For if the nerves are cut in the abdominal cavity of the cat the central end has but a short distance to grow to reach the adrenal. It has been shown by Langley that nerve fibers in the cervical sympathetic regenerate at a fairly rapid rate. If this rate holds for the splanchnics it should not take many days for some of the fibers to reach the adrenal, the distance is so short. That such regeneration takes

place is indicated by our observations on the return of the denervated eye reactions to fatigue. Examination of our animals, in which we have suspected nerve regeneration, has shown that nerve fibers have again connected with the adrenal."

In other words, the assumption that the adrenal secretory fibers regenerate in a relatively short time is used to support the assumption that the denervated eye reaction is due to increased output of epinephrin, and the occurrence of the eye reaction is used to prove that the nerve fibers rapidly regenerate. The only attempt at actual proof of the latter hypothesis is that they see the new nerve fibers with the naked eye in the scar tissue on opening up the animals. No neurologist will accept this as proof. Had the statement occurred only once it might have been considered a slip of the pen. But it is repeated again and again. As to the argument that certain preganglionic fibers in the cervical sympathetic regenerate rapidly, it is well known that a great difference exists in the time of regeneration in different nerves. In the case of certain autonomic fibers of the vagus (cardio-inhibitory fibers, e.g.) it may take years. It is not surely known whether the epinephrin-secretory fibers in the splanchnic or some of them, join nerve cells on their way to the adrenals, in the semilunar ganglion, e.g., or whether they are to be considered as preganglionic fibers right down to the gland, where numerous nerve cells exist. Elliott (23) has suggested that the secretory path may be entirely preganglionic, since there is good evidence that the chromaffin cells of the medulla are ancestrally sympathetic ganglion cells. If there are cell stations for any of the fibers in the semilunar ganglion, regeneration of the post-ganglionic fibers could not occur in the animals in which Hartman and his associates cut out the ganglion.

It is, however, simply a waste of time to speculate on this question; the only way of getting information is to see whether the return of function of the fibers can be demonstrated by an unequivocal method, such as stimulation of the splanchnic above the scar and estimation of the epinephrin given off, or estimation of the spontaneous output of epinephrin after a given time has elapsed. Six years ago we published some results obtained in this way, which, although they do not settle the question whether regeneration can occur, indicate that it may often require a considerable time if it does occur.

For instance, the right adrenal was excised and the nerves of the left cut in a monkey (Macacus rhesus). The animal was allowed to live more than $9\frac{1}{2}$ months. It was indistinguishable in any way from the normal monkeys, was active and mischievous, and became a pet in the laboratory. At the end of the time mentioned, being in perfect health, it was sacrificed to permit estimation of the epinephrin output. The concentration in the adrenal vein was no more than 1:70,000,000, and the output of epinephrin, determined under urethane, was no more, at most, than 0.00002 mgm. per kgm. per minute, i.e., not more than $\frac{1}{10}$ or $\frac{1}{12}$ of the average output seen in the few normal monkeys studied, and in cats and dogs. The rate of blood flow through the adrenal was good, 12.45 grams being collected in 2 minutes. The right adrenal at the time of the operation weighed 0.260 gram, and contained 0.48

mgm. epinephrin. The left adrenal at death weighed 0.292 gram, and contained 0.44 mgm. epinephrin, practically the same as before the operation, indicating that during the experiment under urethane there had been little, if any, depletion of the epinephrin store, and that probably therefore the nerves had not regenerated to any appreciable extent.

In a cat (1), (24), 105 days after removal of the left adrenal and section of the nerves coming to the right semilunar ganglion, the output was not more than $\frac{1}{60}$ of the ordinary average output, estimated by the intestine segment method. In another cat the right adrenal was removed and the nerves coming to the left semilunar ganglion severed: 105 days later the output was found to be at most $\frac{1}{50}$ of the normal average. Section of the left splanchnic in the thorax slightly diminished, and stimulation of it slightly increased the dilatation of the pupil of the denervated eye caused by release of adrenal blood collected in a cava pocket for a definite time. But it was not known whether this was due to regenerated fibers or to fibers which had escaped section. In a third cat the same operation was performed, and after 106 days the output was not more than $\frac{1}{45}$ of the normal average. Section or stimulation of the left splanchnic in the thorax did not affect the output.

In a cat, 65 days after the operation, no epinephrin output was demonstrated, although the intestine segment could have detected $\frac{1}{120}$ of the normal average output. In another cat, after 61 days, no epinephrin was found, although $\frac{1}{60}$ of the normal output could have been detected. After 39 days in another cat, the output was only $\frac{1}{80}$ of the normal average; in another, after 35 days no epinephrin was found, although $\frac{1}{120}$ of the normal output could have been demonstrated. In a cat 20 days after the operation no epinephrin was found, although $\frac{1}{1000}$ of the normal average could have been detected, and so on. In one cat, after 61 days, $\frac{1}{5}$ of the normal average output was found This was the only animal in which the possibility of appreciable regeneration seemed to be present. But even here it was not certain. These are more pertinent facts as regards the question under discussion than anything Langley (or more recently Tsukaguchi (25) in Schafer's laboratory) may have found in the cervical sympathetic; and they do not indicate that the assumption of Hartman, Waite and Powell, that regeneration of the epinephrin-secretory fibers had occurred in their experiments whenever there was dilatation of the pupil of the denervated eye during work, has any basis.

As regards their first assumption, that this dilatation indicates increased epinephrin output, it appears to be necessary to point out again that even if it were true, which it is not, that the pupil of the denervated eye cannot be caused to dilate, after removal of one adrenal and denervation of the other, by influences which cause it to dilate in the

normal animal, unless regeneration of the epinephrin-secretory fibers has occurred, all that this would prove is that epinephrin is necessary for the reaction. Whether an increased output is necessary could only be shown by measuring the output, and this cannot be done by simply looking at the denervated eye.

SUMMARY

The average epinephrin output, measured by the rabbit intestine segment method on a new series of 59 cats is 0.000227 mgm. per kgm. of body weight per minute (expressed as adrenalin base). For a previously published series of 44 cats the average output is the same (0.000225 mgm. per kgm. per minute). For the 103 cats (anesthetized some with ether, some with urethane) the average is 0.000226 ± 0.000007 mgm. per kgm. per minute.

A chart is given showing the grouping of the separate determinations, which are so distributed about the mean as to permit the deduction of a satisfactory average. Nearly 85 per cent of the outputs lie between 0.00010 mgm. and 0.00030 mgm. per kgm. per minute.

The average for 60 cats anesthetized with ether is 0.000207 mgm. and for 43 cats anesthetized with urethane, 0.000252 mgm. per kgm. per minute.

The average output per kgm. per minute for 32 dogs is 0.000227 ± 0.0000096 mgm. per kgm. per minute.

The average output for 71 female cats and dogs is 0.000235 mgm., and for 64 male cats and dogs 0.000218 mgm. per kgm. per minute.

The close agreement in the results of our two extensive series of measurements on cats indicates that the rabbit intestine segment method, when properly employed, is a good method, and that the epinephrin output which we have estimated is not an artificially stimulated output due to the experimental conditions (anesthesia, trauma, etc.). There is no evidence that these conditions have any material influence upon the output. Statements to the contrary, based upon erroneous interpretations of such reactions as dilatation of the pupil of the denervated eye or changes in the rate of the denervated heart, without quantitative estimations of the output, have no real foundation. The same is true of the statement that the physiologically "secreted epinephrin plays a very important rôle in increasing muscular work and delaying the onset of fatigue."

It has been demonstrated that in the rabbit intestine method the defibrination of the blood and the keeping of it (on ice) for the time necessary for assaying the epinephrin do not entail a detectable loss of epinephrin.

BIBLIOGRAPHY

(1) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1917, x, 1.
(2) STEWART AND ROGOFF: This Journal, 1919, xlviii, 397.
(3) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1919, xiii, 108.
(4) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1919, xiii, 183.
(5) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1919, xiii, 303.
(6) STEWART AND ROGOFF: This Journal, 1920, lii, 521.
(7) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1916, viii, 479.
(8) HARTMAN, MCCORDOCK AND LODER: This Journal, 1923, lxiv, 1.
(9) STEWART AND ROGOFF: Journ. Exper. Med., 1917, xxvi, 632.
(10) STEWART AND ROGOFF: Journ. Exper. Med., 1917, xxvi, 637.
(11) CANNON AND HOSKINS: This Journal, 1911, xxix, 274.
(12) CANNON AND DE LA PAZ: This Journal, 1911, xxviii, 64.
(13) GLEY AND QUINQUAUD: Compt. rend. Soc. Biol., 1921, lxxxiv, 706.
(14) STEWART AND ROGOFF: This Journal, 1923, lxiii, 436.
(15) ANDERSON: Journ. Physiol., 1905–6, xxxiii, 156.
(16) JEGOROW: Arch. Slav. de Biol., 1887, iii, 322.
(17) RAPPORT: This Journal, 1922, lx, 461.
(18) HARTMAN, WAITE AND POWELL: This Journal, 1922, lx, 255.
(19) STEWART AND ROGOFF: Journ. Pharm. Exper. Therap., 1922, lix, 87.
(20) BATELLI AND BOETTA: Compt. rend. Soc. Biol., 1902, liv, 1203.
(21) KELLAWAY AND COWELL: Journ. Physiol., 1922, lvii, 82.
(22) BOINET: Compt. rend. Soc. Biol., 1895, xlvii, 273, 325.
(23) ELLIOTT: Journ. Physiol., 1913, xlvi, 285.
(24) STEWART AND ROGOFF: This Journal, 1917, xliv, 543.
(25) TSUKAGUCHI: Quart. Journ. Exper. Physiol., 1916, ix, 281.

ON THE FUNCTION OF THE CEREBRAL VENTRICLES

I. Demonstration of Small Changes in the Capacity of the Lateral Ventricles of the Brain

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In repeating some of the experiments of Dandy (1) in which the production of an internal hydrocephalus by obstruction of the aqueduct of Sylvius has been so admirably demonstrated, the method of freezing the animal in situ and making frozen cross sections of the cranium and its contents was adopted in this laboratory as a means of post-mortem study. The reasons for using this method in preference to others were, first, because the winter climate in this province is well suited for fairly rapid solidification by freezing; secondly, because we thought that this method would give the relations of the intracranial contents uncomplicated by any unequal shrinkage of tissue as is produced by the ordinary fixatives; thirdly, because the "closed box" character of the coverings of the central nervous system would be least likely to be disturbed when this method was used, as there would be no escape of cerebrospinal fluid through mechanical interference; and finally, because it was held that the mechanical disturbance of the relationship between brain, membranes and bone would be reduced to a minimum.

When this method was applied in the case of normal animals so as to establish a basis of comparison with the cranial relationships found in animals which had been rendered hydrocephalic, it was found that a considerable difference existed between Dandy's depiction of the normal and our own results. In figure 2 of the paper by Dandy (1) it will be seen that the lateral ventricles, cut across about the level of the genu of the corpus callosum, are quite large, the walls being 4 mm. apart. The median sulcus varies from 1.5 to 2.5 mm. in width. In our series, in cross sections taken at approximately the same level (fig. 1), the ventricles are found to have their walls practically in apposition. The median sulcus is less than a millimeter in width; the other sulci vary from apparent 0 mm. to about 1 mm. as measured from the photograph.

A number of measurements were taken directly from the frozen sections, working out in the cold and using a micrometer scale and an ordinary low power objective with reflected light. These measurements show values very much smaller. The ventricles in normal dogs, cut across roughly about the level of the pineal body, show the ventricular walls separated by from 2 mm. to 4 mm. at their points of widest divergence. The longitudinal sulci vary in width, according to these measurements, from 0.085 mm. to 0.34 mm. The other sulci are of the same order of magnitude and show similar limits of variation.

It is the purpose of this paper to point out that by using this method of freezing the intact animal and then taking gross cross sections of the brain definite dilatations of the ventricles can be shown to occur, after certain operative procedures, in much shorter periods of time than has generally been supposed possible. It is necessary to point out, however, that for this purpose animals of a similar age, preferably within one year, should be selected. It has been observed in the course of this work that with increasing age there is a tendency to increased size of the ventricles. This tends to confirm previous observations. Unilateral enlargements are not uncommon.

Methods. Various operative procedures were employed in attempting to alter the amount of ventricular fluid. These will be detailed later. After these procedures were completed the animals in all cases were killed and then set out to freeze. Two methods of freezing were employed, natural and artificial.

The natural method was perhaps the more satisfactory, as with our Manitoba winter conditions it permitted of more rapid freezing than did the artificial method. Temperatures of from 0°F. to −30°F. were obtained, and the animals hardened quite rapidly, no crystallization being apparent to the naked eye in the cross sections. Cooling was materially hastened by soaking the animal's fur with cold water, and also by splitting the scalp and stripping the temporal muscles away from the cranium. The animal's mouth was always kept widely open with a gag. A breezy place was also found a useful factor in hastening the process.

In the artificial method the animals were "dressed" in the manner above described, except that they were covered with a jute sack. They were then placed in a commercial cold storage plant. They were here subjected to temperatures of about 0°F. for several days. The animals at the end of this time were well frozen indeed, but there was considerable crystallization observed on section. This would indicate slower freezing by this method than was obtained by use of the natural method. Employees at the plant state that a quarter of beef freezes through in 24 hours under similar conditions.

The hair and skin of the frozen head were removed and the animals were then fixed in a vice and sections cut with an ordinary meat-saw. Vertical transverse sections were taken every half-inch commencing 2 inches in front of the occipital protuberance and working caudalwards. In large and small animals an attempt was made to adjust these sections at corresponding levels, having regard for the relatively small variations that occur.

The sections may then be cleared by one of two methods. They may be flushed at once in a stream of cold water to wash away the debris. If the sections have been cut while under sufficiently low temperature conditions, this will be all that is necessary. But if the cutting is done without sufficiently low temperatures, below 0°F., a greasy envelope tends to form on the cut surface, and this it is hard to get rid of by simple flushing with water without at the same time thawing too much of the subjacent tissue. In these cases it was found best to use a sharp straight-edged knife or broad chisel to cut away this envelope. If the sections can now be photographed at once excellent differentiation may be obtained. Furthermore by working in temperatures not warmer than −3°C. the sections may be studied in this frozen condition. They may be kept for future reference by fixing in 10 per cent formalin solution which has been chilled to −3°C. If the temperature is allowed to rise very slowly, very little distortion will take place aside from that due to the fixative itself.

A. Attempts were made to produce internal hydrocephalus by obstructing the aqueduct of Sylvius following the method outlined by Dandy. Some of these animals died at various times following operation and on being treated postmortem in the manner above described were found to show an interesting gradation in the sizes of their lateral ventricles roughly proportionate to the length of time they had survived.

In these cases the general procedure consisted in doing sub-occipital decompression in dogs anesthetized with ether. The hole was made with a trephine in the squamous portion of the occipital bone in the middle line. This was enlarged with rongeurs down into the foramen magnum. The dura and subarachnoid were then opened by a longitudinal incision. A considerable gush of cerebrospinal fluid was here always encountered. This was mopped up and the fourth ventricle was then opened by snipping through its roof. A small cotton plug mounted on a suitably curved probe was then passed up through the fourth ventricle into the aqueduct, the vermis of the cerebellum being retracted meanwhile. In some cases a gelatine capsule was used as a cover for the cotton plug. After insertion of the obstruction all bleeding was stopped and the margins of the dura were laid together. The

muscles of the neck were then brought together loosely with sutures and the skin wound closed. This was then suitably bandaged and the animal permitted to recover.

The first figure of this series presented is that of an animal in which the Sylvian aqueduct was blocked (fig. 2). This animal died a few minutes after the plug was inserted. It will be observed that the ventricular walls are not quite collapsed. As there was always loss of more or less cerebrospinal fluid when the meninges were opened, one might have expected to find a corresponding shrinkage in the capacity of the ventricles. The ventricles are slightly smaller than is usual, but they are by no means collapsed. In animals that were decapitated prior to freezing the ventricles were found in a similar, perhaps slightly more, collapsed condition.

The second case is that of a normal dog or control (fig. 3). This animal was killed with an overdose of ether without any cranial operation and was then set out to freeze.

The third case (fig. 4) is that of a dog whose aqueduct was obstructed with a cotton plug enclosed in a gelatine capsule. Aside from the preliminary gush of fluid there was, after the operation, practically no drainage of cerebrospinal fluid from the occipital wound. Death took place 28 hours after operation, and shortly after it had been given water by stomach tube. This animal froze rapidly on exposure. The ventricles are appreciably dilated. Considering the age of this animal one would regard this degree of dilatation as rather beyond the limits of normal variation.

The next figure presented shows still more marked dilatation of the lateral ventricles (fig. 5). The aqueduct of Sylvius was obstructed in the usual manner, except that the cotton plug was not covered with a gelatine capsule. The plug, however, had probably become more or less impermeable, since during manipulation it had become soaked with blood and this had clotted. After loose closure of the occipital wound there was practically no oozing of cerebrospinal fluid. The animal did well for the first 24 hours except that it presented a gradually increasing pulse rate. It died 42 hours after operation. It will be seen in the figure that there is a slight but distinct advance in the degree of dilatation of the ventricles as compared with those in the previous figure.

The last case of this series is that of an animal whose aqueduct was plugged in the usual manner and which lived for about 60 hours after the operation (fig. 6). The animal seemed to do quite well at first, was able to move around with some difficulty. Fluids were limited during the first 24 hours. The figure presented (fig. 6) shows a well-marked enlargement of the lateral ventricles. This dilatation was traced through the third ventricle and aqueduct to a point where the aqueduct

is occluded by a fibrinous mass just anterior to the cotton plug. The left ventricle shows somewhat greater dilatation than the right, but both are dilated. There is not as sharp a distinction between the white and grey matter of the brain as usual. But the sulci and gyri do not show any apparent change in size or in shape.

From the progressive enlargement of the ventricular spaces shown by these cases it would seem that it is possible to show definite enlargement of the ventricles within 60 hours of operations in which the aqueduct of Sylvius has been obstructed, even the obstructing material has itself been more or less permeable.

B. It was thought that it would be interesting if some other means could be found of varying the amount of the ventricular contents.

Weed and McKibben (2) observe that following an injection intravenously of hypertonic solutions there occurs a marked diminution of cerebrospinal fluid pressure. This fall in pressure was found to be accompanied by an actual shrinkage in the volume of the brain itself (3). These observers were unable to satisfy themselves that an actual absorption of cerebrospinal fluid took place, accounting for the diminished fluid pressure. If, however, such abstraction of fluid from the cerebrospinal system did occur, we considered that it would be demonstrable by a decrease in the ventricular spaces as shown in frozen cross sections. With this object in view intact dogs were injected intravenously under ether anesthesia with 50 cc. of warmed 33 per cent sodium chloride solution. The injection took, on an average, 25 minutes to perform. Half an hour after completion of the injection the animals were killed with an overdose of ether and set out to freeze in the usual manner,—all meninges intact.

The results obtained were quite surprising. It had been hoped to secure the more or less complete collapse of the ventricular spaces by means of these hypertonic injections. The cross sections of the frozen brains, however, showed that not only were the ventricles not collapsed, but that they were actually quite widely dilated. Figures 7 and 8 present the results obtained. These represent a fair average of the results obtained in eight out of ten dogs.

Discussion. These experiments on the effect of obstructing the drainage from the cerebral ventricles through the aqueduct of Sylvius confirm the results obtained by Dandy. The impression gained, however, from a perusal of Dandy's work is that such changes in the size of the lateral ventricles require a long period of time to induce to a demonstrable degree. Dandy (3) apropos of a case of thrombosis of the vena magna Galeni and the sinus rectus reported by Magendie in 1825, states "The symptoms were only of a week's duration, an interval too short to give demonstrable dilation of the ventricles. As demonstration of excess quantities (of cerebrospinal fluid) are untrustworthy,

the diagnosis of early hydrocephalus may be questioned." From 1 to 3 months were required to produce the gross dilatations reported by Dandy himself in his paper. Our results indicate that ventricular dilatations may be unmistakably demonstrated in a much shorter time, a matter of hours, with proper selection of animals as to age, and by use of the frozen section method. That is to say, the frozen section method permits of the detection of much smaller variations in size of the ventricles than has generally been considered possible.

Our discovery that the ventricles show an increase in size following the intravenous injections of hypertonic salt solutions indicates that the ventricles are capable of change as a result of alteration in the volume of the brain substance. It seems evident that this dilatation takes place as a result of factors other than obstructive in nature. Previous methods employed to produce ventricular distention have depended upon the production of an increased positive pressure within the ventricles by one means or another, such as obstructing the outflow of the cerebrospinal fluid, or by causing an increased production of the fluid, possibly combined with a consequent obstruction to its outflow, by inducing an edematous condition of certain portions of the brain as by ligaturing the vena magna Galeni, as done by Dandy. In our experiments the ventricles are obviously more or less passively dilated as the result of the diminished brain volume. This would seem therefore to constitute a method of inducing ventricular dilatation, though of comparatively slight degree, based on a different and, so far as we can ascertain, a new principle.

We wish to take this opportunity of acknowledging the surgical assistance kindly rendered us by Dr. Oliver S. Waugh in the early part of this work.

SUMMARY AND CONCLUSIONS

1. The normal ventricles in the dog are largely potential cavities, the walls being mostly in apposition except in the case of the lateral ventricles where they are usually separated by 2 to 4 mm. of cerebrospinal fluid intervening between the roof and floor of the main ventricles.

2. By using the frozen section method small changes in the state of distention of the ventricular cavities can be demonstrated.

3. Demonstrable dilatation of the ventricles occurs 28 to 60 hours after obstruction of the aqueduct of Sylvius.

4. Demonstrable dilatation of the ventricles occurs half an hour after the intravenous injection of hypertonic salt solutions.

5. The ventricles are capable of change in size as a result of changes in the volume of the brain substance.

BIBLIOGRAPHY

(1) DANDY: Ann. Surg., 1919, lxx, 129.
(2) WEED AND MCKIBBEN: This Journal, 1919, xlviii, 512.
(3) WEED AND MCKIBBEN: This Journal, 1919, xlviii, 531.

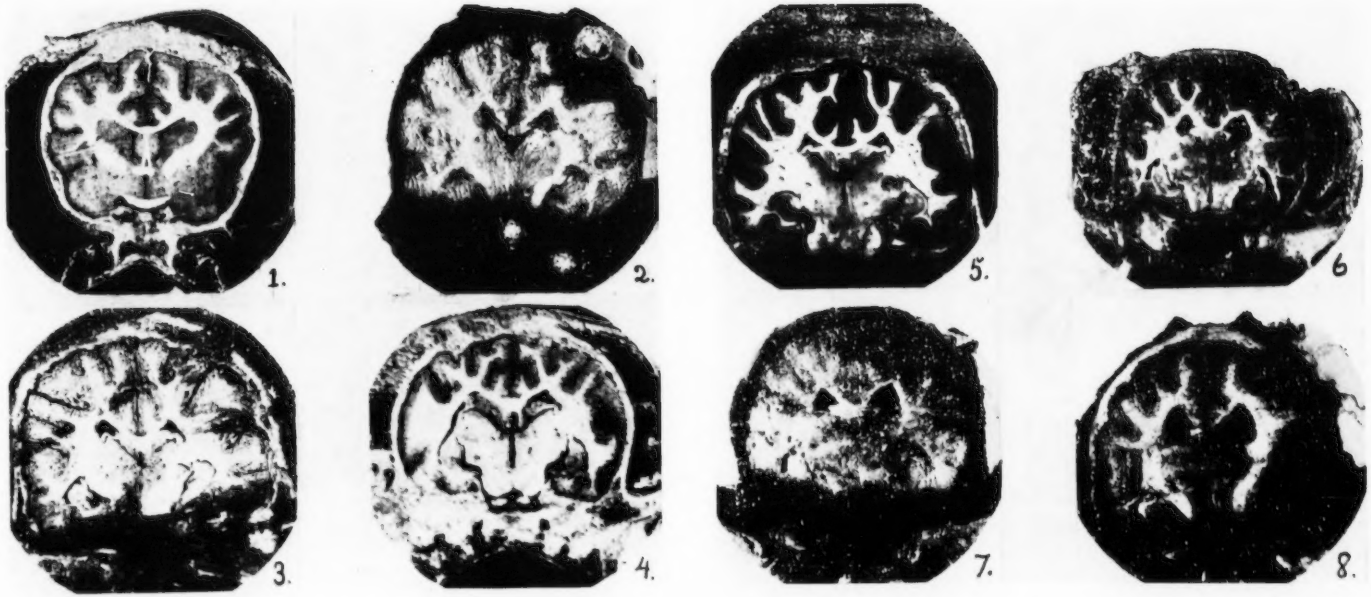

Fig. 1. Section from a normal dog, taken slightly caudad to the level of the genu of the corpus callosum.

Fig. 2. Section from an animal that had lost considerable cerebrospinal fluid during operative procedures, and which had died a few moments after the plugging of its aqueduct.

Fig. 3. From a normal control animal.

Fig. 4. Section from a dog that survived for 28 hours after the obstruction of the aqueduct of Sylvius with a gelatine covered cotton plug.

Fig. 5. Section from a dog that survived for 42 hours after the plugging of the aqueduct.

Fig. 6. Section from a dog that survived for 60 hours after the plugging of the aqueduct.

Figs. 7 and 8. Sections of the brains of dogs that had received intravenous injections of hypertonic salt solution, the injection having been completed half an hour before death. Observe the size of the lateral ventricles.

THE RÔLE OF ANOXEMIA IN THE CAUSATION OF TETANY DURING HYPERPNEA

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In recent literature there have been numerous references to the theory that the symptoms of tetany in forced breathing are due to an increased stability of oxyhemoglobin and a consequent anoxemia in the tissues. This is based on the fact that the combining power of hemoglobin with oxygen may be markedly altered by variations in the carbon dioxide tension, as has been so admirably shown by Bohr, Hasselbach and Krogh and by Barcroft and others. As the carbon dioxide tension of blood is increased the oxygen dissociation curve becomes flattened out. The result of this is that, at any given percentage saturation of hemoglobin with oxygen, the oxygen tension becomes lowered as the carbon dioxide tension is lowered.

In forced breathing there is a very marked decrease in the carbon dioxide tension, and undoubtedly a corresponding change in the dissociation curve of hemoglobin. Reasoning from this it might be concluded that under the conditions of hyperpnea, with a rapid decrease in the carbon dioxide tension, the union of hemoglobin with oxygen would become so firm that an actual tissue asphyxia would result, and that although the hemoglobin in the capillaries might have an abundant supply of oxygen, it would be unable to part with it to supply the needs of the tissues. As a matter of fact, Macleod (1) discusses the phenomena of forced breathing as being the result of anoxemia.

Since the theory of anoxemia in tetany of hyperpnea is purely theoretical, the present investigation was undertaken to obtain data on this point.

EXPERIMENTAL. Two principal methods presented themselves in the study of the rôle of anoxemia in tetany of hyperpnea. The first concerned the oxygen consumption of the entire body as determined by an analysis of the respiratory exchange, and the second concerned the utilization of oxygen from the blood as determined by a study of the venous oxygen unsaturation.

1. Respiratory exchange. The principle of this method of study was to compare the oxygen consumption of the body during hyperpnea and

tetany with oxygen consumption during hyperpnea without tetany. The muscular exertion of deep breathing would naturally cause a certain increase of the oxygen consumption above the basal level, and it would therefore not be permissible to compare the oxygen consumption during hyperpnea and tetany with the basal oxygen consumption. It is possible, however, to undergo the muscular exertion of hyperpnea and at the same time prevent any washing out of carbon dioxide, and hence prevent the occurrence of tetany, by inspiring a 5 per cent mixture of carbon dioxide in air during the hyperpnea. The oxygen consumption under these circumstances then serves as a base level with which to compare tetany. The ideal experiments would be those in which the subject had the same minute volume of respiration when over-ventilating with out-side air as he did when over-ventilating with the 5 per cent carbon dioxide mixture. The exertion of deep breathing would then be the same in both instances.

The carbon dioxide mixture was made up by adding approximately 5 per cent of pure carbon dioxide to air in a large Tissot spirometer. The subject was kept in the recumbent position and requested to make no movements other than those of breathing. The type of mouth-piece and nose-clip used in routine basal metabolism determinations was adjusted, with valves so arranged that the carbon dioxide mixture was inspired and the expired air was collected in a second spirometer. The subject then began the deep breathing, keeping the rate of respiration constant at eighteen per minute by means of a loud metronome, and making each respiration maximal. After washing out the tubing and second spirometer with expired air, the collection of expired air was begun. The period usually lasted about three minutes before all of the carbon dioxide mixture was used up, time being kept with a stop-watch. The minute volume of expired air was then calculated, samples of both the inspired carbon dioxide mixture and of the expired air were taken, and later analyzed in duplicate for oxygen and carbon dioxide in the Haldane gas analysis apparatus. The oxygen and carbon dioxide content of the inspired air were corrected on the basis of the expired nitrogen, and the minute consumption of oxygen and "production" of carbon dioxide calculated. During the period of hyperpnea with carbon dioxide there were never any symptoms of tetany. After the completion of this first period the subject was given a few minutes' rest, and then the mouth-piece was readjusted so that he was inspiring out-door air. He then began hyperpnea at the same rate and depth as before. After definite symptoms had developed, i.e., tingling in the extremities, stiffness in the facial muscles, and positive Chvostek sign of tetany, usually in from three to sixteen minutes, collection of the expired air was begun. The minute volume of expired air was again

calculated, duplicate analyses of oxygen and carbon dioxide in the expired air made, and the oxygen consumption and carbon dioxide production calculated.

The results of these experiments are given in table 1. It will be seen that in three experiments the minute volume of ventilation was practically identical in the two periods, so that the muscular work of deep breathing was the same in both instances. The respiratory quotient showed a very marked increase during hyperpnea with air, and the oxygen consumption was definitely increased. In the fourth experiment the minute volume of ventilation was greater while carbon dioxide was being inspired than in the second period, so that the muscular work of

TABLE 1

EXPERIMENT NUMBER		OXYGEN CONSUMPTION PER MINUTE	CARBON DIOXIDE PER MINUTE	R.Q.	VENTILATION PER MINUTE	INSPIRED AIR		DURATION OF HYPERPNEA WITH AIR BEFORE STARTING
						O_2	CO_2	
		cc.	*cc.*		*liters*	*per cent*	*per cent*	*minutes*
1	During hyperpnea with CO_2	290	203	0.70	25.1	19.85	4.51	
	During hyperpnea with air	326	497	1.53	25.2	20.93	0.03	5
2	During hyperpnea with CO_2	363	403	1.18	39.5	19.95	4.24	
	During hyperpnea with air	513	708	1.38	39.8	20.93	0.03	16
3	During hyperpnea with CO_2	242	141	0.58	19.8	19.84	4.98	
	During hyperpnea with air	272	388	1.43	19.4	20.93	0.03	5
4	During hyperpnea with CO_2	439	489	1.11	55.6	19.92	4.42	
	During hyperpnea with air	434	799	1.84	40.1	20.93	0.03	5
5	During hyperpnea with CO_2	311	292	0.94	49.4	19.93	3.93	
	During hyperpnea with air	375	871	2.32	55.1	20.93	0.03	3

breathing was greater in this period. Nevertheless, the oxygen consumption was essentially the same in the two periods. Presumably it would have been lower in the first period had the volume of respiration been the same as in the second period. In the fifth experiment these relations are reversed, the ventilation being greater in the second period, and the oxygen consumption markedly increased. In all of the experiments except no. 2 there were no marked tetanic contractions of the muscles, such as spasm of the hands, but there were definitely positive Chevostek signs. That is, tapping the side of the face caused a spasmodic contraction of the muscles about the mouth and eyes. There was also marked tingling and numbness in the extremities, and "stiff-

ness" in the facial or other groups of muscles. The increased oxygen consumption was therefore probably due to increased tonicity of muscles. In experiment 2, after hyperpnea for sixteen minutes, there was definite spasm of the facial muscles, with difficulty in articulation, and tightness of the chest and abdominal muscles, and slight spasm of the hands. The oxygen consumption was accordingly much more markedly increased above the control level in this experiment than it was in the others. This would seem to indicate that as the tetany increases in severity the oxygen consumption increases, and that as in all forms of muscular contraction, oxygen is used during the muscular contraction in tetany.

The alveolar carbon dioxide tension was studied by the Plesch bag method in experiment 2, the percentage of carbon dioxide being determined in the Haldane apparatus. The tension at rest was 39.3 mm. of mercury, and at the end of the period of hyperpnea with air it had fallen to 22.6 mm.

2. Blood oxygen analyses. If oxyhemoglobin is so stable during hyperpnea and alkalosis that it cannot readily give up oxygen to the tissues, it should return through the veins still containing a large percentage of oxygen. This was studied in the following way. After the subject had rested for a short time in the recumbent position a sample of blood was drawn without stasis and under oil from the anti-cubital vein (2). He then underwent hyperpnea as in the first series of experiments, inspiring a mixture of 5 per cent carbon dioxide in air. After four or five minutes of this another sample of blood was drawn from the same vein, and the deep breathing discontinued only when the drawing of the sample had been completed. After this the hyperpnea was again started, this time inspiring room air, so that symptoms of tetany soon developed. When these symptoms were well established, a third sample of blood was taken. Each sample was then immediately analyzed in duplicate for oxygen content and oxygen capacity in the modified Van Slyke apparatus by the method of Van Slyke and Stadie (3). In three of the four experiments the carbon dioxide capacity of the plasma was also determined in duplicate. Table 2 shows the results of these experiments. In three of them the mere muscular exertion of hyperpnea was accompanied by an increase in the oxygen unsaturation of the anti-cubital venous blood. This corresponds with observations reported by Lundsgaard and Moller (4), who found that muscular exercise of the legs was usually accompanied by an increased oxygen unsaturation in the venous blood from the arm, and as suggested by them is presumably due to slower blood through the arm as a result of vasomotor adjustments of the circulation to exercise. The fourth subject showed a slight decrease of the venous oxygen unsatura-

tion during hyperpnea with carbon dioxide, and possibly corresponds to subject 17 in the Lundsgaard and Moller experiments, in whom there was a similar reaction to exercise. During hyperpnea with air and while there were distinct symptoms of tetany, the venous blood in three instances showed a further increase in the oxygen unsaturation. This indicates that the tissues were certainly extracting oxygen from the hemoglobin during the conditions of alkalosis and tetany, and in reality extracting more oxygen than under normal conditions of acid-base equilibrium, probably in order to meet the increased oxygen consumption of the tissues during tetany. Obviously the lowering of the carbon

TABLE 2

EXPERIMENT NUMBER		OXYGEN BOUND BY HEMOGLOBIN IN VENOUS BLOOD	OXYGEN CAPACITY	PERCENT SATURATION OF VENOUS BLOOD	OXYGEN UNSATURATION	CARBON DIOXIDE CAPACITY OF PLASMA	DURATION OF HYPERPNEA BEFORE BLOOD WAS TAKEN
		volume per cent	*volume per cent*		*volume per cent*	*volume per cent*	*minutes*
6	At rest	12.74	18.41	69.2	5.67	68.4	
	During hyperpnea with CO_2	11.24	18.81	59.8	7.57	68.5	1½
	During hyperpnea with air	9.57	19.64	48.7	10.07	64.2	15
7	At rest	16.31	19.95	81.7	3.65	61.8	
	During hyperpnea with CO_2	16.78	20.12	83.4	3.34	61.0	5
	During hyperpnea with air	16.06	19.92	80.6	3.86	57.0	8
8	At rest	12.18	19.03	64.0	6.85	70.6	
	During hyperpnea with CO_2	12.25	19.22	63.7	6.97	73.6	3
	During hyperpnea with air	15.23	20.55	74.1	5.32	64.1	6
9	At rest	15.94	*20.55	77.6	4.61		
	During hyperpnea with CO_2	13.20	*20.55	64.4	7.35		3
	During hyperpnea with air	11.91	*20.55	58.0	8.64		5½

* Determined on a mixture of all three samples.

dioxide tension in the blood had not so over-stabilized the oxyhemoglobin as to cause a tissue asphyxia. In the fourth experiment the venous oxygen unsaturation was somewhat less during tetany than during hyperpnea with carbon dioxide, but was still within the normal limits. Increased blood flow in the arm may have feen a factor in this instance, as a result of altered vasomotor conditions. At any rate, hemoglobin was still parting with a good supply of oxygen.

The next factor to be examined was the oxygen saturation of arterial blood before and during tetany of hyperpnea. A resting sample of blood was taken from the radial artery under oil. The subject then

over-ventilated until there were marked symptoms of tetany, and the carbon dioxide tension of the alveolar air had fallen from 44 mm. to 23.2 mm. of mercury. A second sample of arterial blood was then taken. Analyses showed an increase in the saturation of the blood with oxygen, and a decrease in the carbon dioxide capacity of the plasma (see table 3). During tetany of hyperpnea the arterial blood was therefore arriving at the tissues with a full supply of oxygen, and leaving the tissues with more oxygen extracted than normally.

It is interesting to estimate what the tension of oxygen in the blood would be under the conditions of lowered carbon dioxide tension in hyperpnea. In sixteen experiments by Grant and Goldman (5) the alveolar carbon dioxide tension fell from an average of 41.8 mm. to an average of 21 mm., the lowest being 18 mm., during hyperpnea and tetany. Barcroft (6) has published dissociation curves of his own blood showing the difference in the curves at 40 mm. CO_2 tension and at 20 mm. tension. Under normal conditions the arterial blood is approxi-

TABLE 3

EXPERIMENT NUMBER	OXYGEN BOUND BY HEMOGLOBIN IN ARTERIAL BLOOD	OXYGEN CAPACITY	PER CENT SATURATION OF ARTERIAL BLOOD	CARBON DIOXIDE CAPACITY OF PLASMA	ALVEOLAR CO_2 TENSION	DURATION OF HYPERPNEA BEFORE BLOOD WAS TAKEN
	volume per cent	*volume per cent*		*volume per cent*	*mm. of mercury*	*minutes*
At rest	17.25	17.98	95.9	68.0	44 0	
During hyperpnea with air	17.51	17.83	98.2	58.9	23.2	6

mately 95 per cent saturated, and the venous blood may be 65 per cent or even less saturated with oxygen. If these points are plotted on the dissociation curve at 40 mm. CO_2 tension, it is found that the arterial blood has an oxygen tension of 80 mm. and the venous blood an oxygen tension of 34 mm. During hyperpnea and tetany the arterial blood was 98.2 per cent saturated, and the average venous saturation was 65.3 per cent in four experiments in which this was determined. If these points are plotted on the dissociation curve at 20 mm. CO_2 tension it is found that the arterial blood has an oxygen tension of 90 mm. and the venous blood an oxygen tension of 26 mm. The average oxygen tension in the capillaries is therefore approximately the same in both instances. There is some question as to whether the oxygen tension in the tissues is zero or not, but at any rate there is a sufficient margin below the venous oxygen tension of 26 mm. to allow the venous blood to leave the tissues with an oxygen tension either above or in equilibrium with the tissue oxygen tension, and the venous oxygen tension would

undoubtedly be above the intra-cellular oxygen tension of striated muscle.

3. The effect of inhaling oxygen. One more set of experiments was necessary. It has been suggested (7) that oxygen inhalations relieved the symptoms developing during hyperpnea. To test this a subject underwent forced breathing for five minutes, at the end of which time there was slight tingling and numbness in the face and hands, a positive Chvostek sign of tetany, and a tight feeling in the chest and abdomen. The subject then began inhaling pure oxygen, valves being so arranged that there was no rebreathing, and continued the hyperpnea. Instead of being relieved, the symptoms continued to increase, so that within the next minute and a half there was definite spasticity of the facial muscles, more marked Chvostek sign, much more tingling in the extremities, and a positive Trousseau sign of tetany. Another subject underwent forced breathing, inspiring throughout the experiment a mixture of equal parts of oxygen and air (said to relieve the symptoms of tetany). This was continued for five minutes, during which time there developed progressively, numbness and tingling in the face, a positive Chvostek sign, stiffness in the facial muscles and difficulty in articulation, tingling in the hands, and finally a slight spasm of the hands on compressing the arm above the elbow. Therefore, inhaling pure oxygen had no effect whatever in relieving the symptoms of tetany, the symptoms progressing as though air were still being inspired, and when hyperpnea was undergone with a rich oxygen mixture the symptoms developed in the same manner as during hyperpnea with air alone.

Review of literature. Davies, Haldane and Kennaway (7) observed numbness and tingling of the extremities, fibrillary twitching of the orbicularis palpebrarum, and slight Rombergism after forced breathing, and attributed this partly to increased stability of oxyhemoglobin and partly to vasoconstriction. They found that these symptoms were "mostly relieved by the inhalation of a few breaths of pure oxygen." Assuming that increased stability of oxyhemoglobin is a factor in the production of tetany in forced breathing, it is difficult to see what effect inhaling pure oxygen could have on the oxygen supply to the tissues and thus on the tetany, aside from the effect of the very small increase in the amount of oxygen dissolved in the blood, since the hemoglobin is already practically saturated. These authors also thought that there would be comparatively little alkalosis in the tissues, because it has been concluded by Yandell Henderson in numerous papers on acapnia and shock that forced breathing decreases the peripheral circulation. It is undoubtedly true that the loss of carbon dioxide would never be as great in the

tissues as in the blood, because there is presumably some lag in the fall of carbon dioxide tension brought about first in the alveolar air, then in the arterial blood, capillaries and tissues, in the order named, the tissues being the last to be affected. However, Yandell Henderson and Haggard (8), in discussing the decrease of the alkaline reserve of the blood during over-ventilation state that the "simplest supposition on which to explain it is that when the alkalinity of the blood is abnormally increased by blowing off of carbon dioxide, alkali passes out of the blood into the tissues." If this be true, then there must be a very definite alkalosis in the tissues during forced breathing. Bazett and Haldane (9) found that when a subject was immersed in a hot bath, there was definite over-ventilation (respiratory quotient 1.3) and slight symptoms of tetany, relieved by inhaling 8.5 per cent carbon dioxide with 14.2 per cent oxygen, or by equal volumes of air and oxygen. They concluded that the symptoms "were therefore probably due to oxygen want in the tissues caused by over-stability of oxyhemoglobin, as in voluntary forced breathing." When the temperature of the bath was kept constant and the body temperature constant at 38.6°C. the respiratory quotient was 0.81 and there were no symptoms.

Greenwald (10) is of the opinion that the symptoms following large doses of sodium bicarbonate and the symptoms of gastric tetany are not due to an alkalosis but to poisoning with the sodium ion. This is an unsettled question and will not be discussed at present. He also subscribes to the view that it is a tissue anoxemia which is the exciting factor of the symptoms of tetany in hyperpnea, rather than the alkalosis, making the statement that "there can be little doubt but that forced breathing produces tissue asphyxia."

Morris (11) could find no effect on the electrical irritability of the neuromyon when there was an excess of sodium. He found that asphyxia, lowering the body temperature, and anemia, all of which decreased the oxygen supply to the tissues, caused an increased electrical irritability. Administration of cyanides also increased the electrical irritability, but it should be noted that cyanides are powerful respiratory stimulants and the fact that he found a lowered blood carbon dioxide at the time that the electrical irritability was increased indicates that over-ventilation may have played a part in increasing the electrical irritability. Morris also found that injection of sodium carbonate decreased the oxygenation of the arterial blood and greatly decreased the amount of oxygen given up to the tissues. It is conceivable that the injection of large doses of alkali might so depress the respiratory center that respirations would greatly decrease or even cease for a short time, while carbon dioxide was accumulating in the blood to restore the normal ratio of $H_2CO_3 : NaHCO_3$. In this event the percentage of

oxygen in the arterial blood would naturally steadily decrease, until the normal respiration was restored. No note was made concerning the condition of the respiration during or after the injection of the alkali, but it may have been greatly decreased. Morris concluded that anoxemia was the essential condition underlying an increased electrical irritability of the myoneuron.

Koehler (12) has suggested that with the rapid elimination of carbon dioxide from the blood in hyperpnea, sodium ions would pass into the tissue fluids and into the urine, and that hemoglobin would then be left combined with chlorin ions and unable to get rid of them because there would be insufficient sodium available to receive the chlorin ions. The hemoglobin would therefore be unable to form oxyhemoglobin. The anoxemia resulting would stimulate the respiration, more carbon dioxide would be washed out of the blood, and thus a vicious circle established. As a matter of fact, it was found in the experiment noted above that during hyperpnea, at a time when there were definite symptoms of tetany, and an alveolar carbon dioxide tension of only 23 mm., hemoglobin was taking up its full quota of oxygen, the arterial blood being 98.2 per cent saturated with oxygen, and actually more saturated than it had been during normal respiration (95.9 per cent). This increase may have been due to a change in the dissociation curve of hemoglobin, to an increase in the average oxygen tension in the alveoli during hyperpnea, or to a more complete expansion of the lungs and aeration of all the alveoli, or all three of these factors combined.

SUMMARY AND CONCLUSION

1. The literature concerning the theory of a tissue anoxemia in tetany of hyperpnea is reviewed.

2. It has been shown that during the alkalosis and tetany of hyperpnea there is a greater oxygen consumption of the body, as determined by a study of the respiratory exchange, than in a similar hyperpnea unaccompanied by alkalosis or tetany.

3. The oxygen unsaturation of venous blood from the arm was greater during hyperpnea with tetany than in hyperpnea alone in three of four experiments.

4. Hyperpnea with alkalosis and tetany was accompanied by an increase in the oxygen saturation of the arterial blood.

5. Inhalation of pure oxygen had no effect on the development of symptoms of tetany during hyperpnea, and hyperpnea with a mixture of equal parts of oxygen and air resulted in tetany in the same manner as though air alone had been used.

It must be concluded that anoxemia plays no part in the causation of tetany during hyperpnea, and that the lowering of the carbon dioxide

tension of the blood under these conditions is not sufficient to cause a tissue asphyxia.

I wish to express my thanks to Miss Gladys Kauffmann who did the majority of the gas analyses in the Haldane apparatus.

BIBLIOGRAPHY

(1) Macleod: Physiology and biochemistry in modern medicine, 1922, 381.
(2) Lundsgaard: Journ. Biol. Chem., 1918, xxxiii, 119.
(3) Van Slyke and Stadie: Journ. Biol. Chem., 1921, xlix, 43.
(4) Lundsgaard and Moller: Journ. Biol. Chem., 1923, lv, 315.
(5) Grant and Goldman: This Journal, 1920, lii, 209.
(6) Barcroft: The respiratory function of the blood.
(7) Davies, Haldane and Kennaway: Journ. Physiol., 1920–21, liv, 32.
(8) Henderson and Haggard: Journ. Biol. Chem., 1918, xxxiii, 345.
(9) Bazett and Haldane: Journ. Physiol., 1921, lv, p. iv.
(10) Greenwald: Journ. Biol. Chem., 1922, liv, 285.
(11) Morris: Brit. Journ. Exper. Path., 1922, iii, 101.
(12) Koehler: Arch. Intern. Med., 1923, xxxi, 590.

THE RELATIONS BETWEEN LESIONS OF THE BRAIN STEM, WATER ELIMINATION AND BODY TEMPERATURE

(PRELIMINARY REPORT)

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It was shown by Isenschmid and Krehl (1) that destruction of the optic thalamus destroyed the body temperature regulating mechanism in the rabbit. The writer (2) found the same true in birds, and Bazett and Penfield (3) in an admirable study of decerebration in cats recently published, have confirmed this relation. Matthews (4) showed that a source of irritation in the region of the infundibulum may cause polyuria. Several workers have recently shown that hypothalamic lesions may lead to polyuria, although the hypophysis is not damaged. Barbour (5) has recently cited experimental support to the view that shifting of the water balance between blood and tissues is an important factor in the maintenance of normal body temperature, and this shifting is in some way dependent on the central nervous system. Very recently the writer has obtained experimental results which correlate these three factors in a striking manner.

It is a well-known fact that in the pigeon the cerebral hemispheres may be removed and the animal kept alive indefinitely without the loss of the ability to regulate its body temperature provided care is taken not to damage the nerve centers around the third ventricle. If the optic thalamus be destroyed the animals at once become poikilothermous and rapidly lose weight. Certain of these birds may be kept alive for several weeks if the body temperature is artificially maintained by keeping them in a warm incubator. Usually in order to maintain the normal body temperature of the bird at 40°C. it is necessary to keep the incubator at 30°C. For adult birds this is a very constant relation, failing only at a time when nutritional disturbances are indicative of starvation. The 10-degree difference between the environmental temperature and the body temperature is due primarily to the insulating action of the feathers, for if the feathers be removed the body temperature then falls to within 1 or 2 degrees of the environmental temperature.

Several years ago the writer observed the immediate loss of weight that follows loss of this part of the brain. It suggested a profound alteration of metabolism and efforts have been made to explain it. Evidence just obtained indicates that this loss in weight is represented to a major extent by the loss of water from the body.

The polyuria that occurs after destruction of the optic thalamus may be measured by actually collecting the urine in a metabolism cage, or indirectly by measurements of loss of body weight. In order to determine the water loss by determinations of the body weight it is obviously necessary to know the loss of weight due to the respiratory exchange and the total solids of urine and feces.

Determinations of the respiratory exchange have been made. The oxygen consumption and the carbon dioxide excretion in the normal pigeon are increased by feeding and exposure to cold. Under standard conditions of deprivation of food and maintenance of an atmospheric temperature of 28° to 30°C. the oxygen consumption assumes a fairly constant level. It was now found that a determination of the respiratory exchanges of a normal pigeon and a pigeon rendered poikilothermous by destruction of the optic thalamus agree with each other very closely under the following conditions: no food for twenty-four hours and exposure of both to the atmospheric temperature of 30°C., at which both birds have the same body temperature. The respiratory quotient under these conditions for both homothermous and poikilothermous birds lies between the values of 0.68 to 0.78. Calculations of heat production on the basis of the respiratory exchange exhibit similar values for both. In both animals the total solids excreted by the intestinal and urinary tracts are about 1 gram per twenty-four hours. These findings suggest that the loss of temperature regulation is not therefore primarily due to metabolic changes following the brain lesion.

The loss of body weight is due principally to the elimination of water from the body, for with a respiratory quotient of 0.70 to 0.75, the weight of carbon dioxide excreted is almost exactly balanced by the weight of the inspired oxygen under the conditions specified, and the total solids excreted in the normal and in the poikilothermous birds, agree within 1 gram. With these considerations in mind the tremendous loss of water after these brain injuries is indicated in table 1.

In some animals this increased water elimination and progressive loss of body weight are, at a slow rate, extending over days and weeks. Six weeks is, in my experience, the longest period of life attainable after destruction of the thalamus. In contrast to this chronic type, other birds have exhibited an acute polyuria in which as much as 20 per cent of the body weight may be lost within forty-eight hours. The latter condition may be produced with greater certainty by taking care that

the hypothalamic region be damaged. In both types of animals the poikilothermous condition follows immediately after the surgical operation and precedes any great loss of water from the body. Hence the polyuria itself can not be the primary factor responsible for the loss of the ability to regulate body temperature. This consideration does

TABLE 1

The loss of water per 24 hours of normal adult pigeons and poikilothermous pigeons. No food or water for 24 hours preceding the determinations. All animals kept at a uniform atmospheric temperature of 28°–30°C.

ANIMAL	WATER LOSS PER 24 HOURS
	grams
Normal—No. 32	22
Normal—No. 48a	22
Normal—No. 48b	18
Normal—No. 54	17
Poikilothermous—No. 53	40
Poikilothermous—No. 46	34
Poikilothermous—No. 47	48
Poikilothermous—No. 43	58
Poikilothermous—No. 41	62

TABLE 2

Poikilothermous birds showing relations between hyperthermia and the degree of polyuria

ANIMAL	WATER LOSS PER 24 HOURS	PER CENT OF BODY WEIGHT	ATMOSPHERIC TEMPERATURE	BODY TEMPERATURE
Lesser degree of polyuria				
	grams		°C.	°C.
No. 43	32	11	31	38.0
No. 50	34	13	31	41.5
Severe polyuria				
No. 41	62	20	30	44.6
No. 54	75	21	31	45.1

not exclude a water shifting mechanism between blood and tissues, in Barbour's sense, as being an essential factor.

Whether or not the water elimination is that of preformed water in the body, or water produced by metabolic processes, is not at present clear. Studies of blood concentration and urinary analyses will be made, to examine this question.

The preceding considerations now render possible an interesting explanation of certain hyperthermias due to brain stem lesions. If an animal is rendered poikilothermic, obviously a sufficiently high atmospheric temperature will force the body temperature up to the death point. This explanation will account for some recorded hyperthermias. But there is an additional type that may be produced. If an atmospheric temperature be selected such as to maintain a normal body temperature in the poikilothermic animal and this animal exhibits an acute polyuria, the temperature will rise when there has been a sufficient loss of water, somewhat independently of the atmospheric temperature (table 2). This type of hyperthermia in the poikilothermous bird has been observed only in those cases of acute polyuria where the loss of water has been about 20 per cent of the body weight within twenty-four hours.

In all the experimental work, care has been taken to leave the pituitary body intact. There may have been circulatory alterations in the hypophysis due to the brain injuries, and if Hering's views be correct, that the pituitary hormones are secreted into the cerebro-spinal fluid, the brain lesions may have excluded this route of absorption. Hence the possibility of pituitary involvement in the effects described can not be excluded.

BIBLIOGRAPHY

(1) Isenschmid and Krehl: Arch. f. Exper. Path., 1912, lxx, 109.
(2) Rogers: This Journal, 1919, xlix, 271.
(3) Bazett and Penfield: Brain, 1922, xlv, 185.
(4) Matthews: Arch. Int. Med., 1915, xv, 451.
(5) Barbour: Physiol. Reviews, 1921, i, 295.

THE GILL MOVEMENTS IN ONE OF THE PERENNIBRANCHIATE URODELA (NECTURUS MACULATUS) AND THEIR RELATION TO THE CENTRAL NERVOUS SYSTEM

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Many years ago I made observations upon the movements of the external gills in the mud puppy or water dog (*Necturus maculatus* s. *Menobranchus lateralis*) which is easily obtained in this part of North America. The gills of these animals are particularly interesting objects of physiological investigation because of their great development, their persistence throughout life,[1] the ease with which their movements can be observed without subjecting the animal to any constraint, and the fact that they seem to play a much more important part than the lungs as a specialized respiratory mechanism.

The results of the observations were communicated to the Physiological Society in 1895. But as they were only published in the form of a brief reference in a textbook (3), they do not appear to have come under the notice of subsequent writers on the subject (4), (5), (6). In an article on "The otic labyrinth and equilibration in Necturus" in the Jubilee Volume dedicated to Professor Zwaardemaker (13) I have had occasion to refer to these experiments, without being able to give any details. I therefore judge it useful to present here a brief account of the work, which, so far as I am aware, constitutes not only the earliest systematic study of the gill movements in this group but the only one specially concerned with the relation of the movements to the central nervous system.

About twenty specimens, mostly full grown, were used in the observations. They were kept in stock in a large tank with running water, in which were also frogs. The animals which were not subjected to a brain operation lived many months. They were observed to eat the

[1] It is astonishing to find the statement in a standard work of the last century (1) that in *Menobranchus* "the external gills disappear, without being replaced by internal organs of a like kind, and nothing remains of the branchial apparatus, save the fissures leading from the pharynx and the surface of the neck." Longet (2), writing about the same time, states the matter accurately.

frogs' eggs, also tadpoles and probably some of the small frogs. For observation of the gills, and always after operations, they were removed to a small tank with glass sides, supplied with running water. A control normal animal was placed in the same tank as the operated animal.

The operations on the brain were generally done under ether, the animal being held wrapped in a towel, only the head protruding. In one or two cases no ether was given. It was observed that during the operation a copious outpouring of viscid secretion took place from the skin. The skull was opened either with a small trephine or with bone forceps, and the brain exposed. An aseptic operation was of course out of the question; the animals had to be kept in water and eventually the stitches (covered with collodion) came out. Yet the survival period was as long as 4 weeks.

When the cerebral hemispheres only were removed the animal, if a short time were allowed for the ether action to disappear, would right itself if laid on its back. In a few instances this power, while present immediately after the operation, disappeared in the course of 24 hours or more, possibly owing to the destructive effect of water leaking through the stitches or to pressure of the clot. When, in addition to the cerebral hemispheres, most of the mid-brain (optic lobes) was removed the animal lost the power of righting itself for the time it survived (14 days in one instance). After removal of the cerebral hemispheres the animal showed a much smaller degree of spontaneous locomotion than the normal animals, but would move when stimulated by being touched or pinched. Without such stimulation it would sometimes, but only rarely, assume a position rather characteristic of the normal animal, erecting its head and neck (but only slightly) as if "standing on tiptoe" on the forefeet. Reflex movements of a limb were easily obtained on pinching a toe, and often the contralateral limb moved also, although generally not so extensively.

The most interesting observations after removal of the cerebral hemispheres were made on the movements of the gills. The normal animal, under our laboratory conditions, executed rhythmical movements of the gills from time to time, but often they would be found retracted and at rest. The operated animal, on the contrary, moved them almost incessantly for long periods after the operation. Probably after several days there was a tendency for the movements to slacken somewhat, and there might be short intervals of rest. But the contrast with the normal animal was great, in spite of the fact that the latter moved about in the tank much more than the decerebrate animal. This is a striking illustration of the slight dependence of these respiratory movements on the intensity of the metabolism, in contrast to the respiratory movements of higher forms. There is no evidence that

the respiratory function of the skin was lessened after the operation, although it is possible that the blood pressure might have been lowered at first and the speed of the blood, including that passing through the skin, correspondingly diminished. There was always a not inconsiderable hemorrhage in these brain operations. The respiratory function of the lungs is normally so little in evidence in Necturus that there is still less reason for supposing that the greatly increased activity of the gill movements was compensatory for diminished pulmonary respiration. In the operated, as in the normal animals, although probably not so frequently, a bubble of gas has been seen escaping from the mouth as the animal brought its head to the surface of the water. Even in the normal animals this was not very frequently observed, nor was it clearly made out that air was taken in by the mouth and forced into the lungs by a swallowing movement, as Babak (5) saw in the two specimens studied by him. Luckhardt and Carlson (12) state that they "have repeatedly seen these animals (Necturus) attempt to swallow air, the air escaping by the gill slits and in no instance entering the lungs."

Reflex effects upon the gill movements could be obtained after removal of the cerebral hemispheres. Stimulation of the tail or a limb, or the skin in the neighborhood of the gills usually caused a slight inhibition, often only the intermitting of a single contraction. Sometimes when the gills, although protruded, were not contracting, such stimulation caused them to be retracted. Not infrequently when the gills happened to be at rest, stimulation of the animal was seen to be followed by rhythmical and long-continued gill movements.

It should be stated further that although usually after ablation of the cerebral hemispheres the gills on the two sides moved together, this was not invariably the case. The gills on one side, while protruded, might remain at rest even for a long time, while those on the other side were moving rhythmically. Nevertheless the motionless gills might be perfectly capable of movement, as proved by their starting contractions again, perhaps after days of rest. It is not known whether or in what degree, if at all, these phenomena were due to further injury to the brain by entrance of water.

A typical protocol follows.

March 13, 1895. 2:45 p.m. Cerebral hemispheres removed without ether. The animal would not lie on its back. Rate of gill movements 19.6, 22.9, 24.5 and 24.4 per minute at 4:16 p.m., 4:18 p.m., 4:23 p.m. and 4:55 p.m. respectively. The rhythm was regular, and when the animal was left alone in the tank the gills on both sides always moved simultaneously. A touch (from the tail of a normal animal in the same tank) over one set of gills caused them to stop on both sides for one contraction. Then the gills on the two sides moved alternately for a few

contractions as if one of them had lost time. Then the rhythm became simultaneous again. Stimulation of the right posterior limb caused it to be drawn in toward the body, and the tail to bend to the right. When the left leg was stimulated it was drawn in toward the body, and the tail bent to the left. Stimulation of the skin in the head region or just behind the anterior limbs may cause: *a*, inhibition of the gill movements on both sides for a contraction or two: *b*, inhibition of movements of the gills on the same side while they continue on the other side: *c*, loss of rhythm of the movements on the two sides. Stimulation of the gills caused the same effect, but not so markedly.

March 14, 7:45 a.m. Found lying on its back in the tank, the gills moving regularly (66 a minute at 11:50 a.m.). It does not right itself at once if laid on its back, but it can maintain the normal position when put into it, and it makes considerable movements if touched or pinched, moving about for some time, arching its head up towards the surface of the water, and opening the mouth, as does the normal animal. The control was seen to rise to the surface, open the mouth and allow a bubble of gas to escape, but no gas came from the operated animal when it opened its mouth, nor did it attempt to secure or swallow food (a tadpole) when it was put into its mouth. The gills are moving incessantly (57 a minute at 12:16 p.m.). At 4:00 p.m. the movements were slower (23 a minute) and perhaps hardly so regular. Sometimes a movement is double, first a partial or abortive contraction and then a contraction more extensive than ordinary.

March 16, 2:00 p.m. Does not right itself if turned on its back. Responds actively to stimulation (induction shocks). The gill movements are less active, though still more evident than those of the control. A few minutes after stimulation it was seen that regular movements of the left gills were going on (28 a minute). The right gills were perfectly motionless, although protruded. Mechanical stimulation of the right or left gills, or of the skin over them or below the fore legs had no effect. At 3:47 p.m. the left gills were moving as before (29 a minute). The control lies covered in the sand at the bottom of the tank, but the operated animal lies on the top of the sand. Weather cold. Temperature of water 11.3°C.

March 17, 2:49 p.m. The operated animal, as always, was found in the natural position; it had shifted its place in the night, as had the control, and from their position they could not be distinguished from each other. But the gills of the control are motionless, while the left gills of the operated one are moving vigorously at the rate of 26 a minute. The right gills are at rest. Mechanical stimulation of the tail (with the thermometer) caused an immediate acceleration of the left gills (to 38 a minute), and the movements became as regular in rate and extent as on the first day after the operation, whereas before the stimulation there was an occasional double movement, first a slight contraction immediately followed by a larger one, or a contraction would appear to be missed altogether. The right gills remained continuously protruded but motionless, and stimulation of the tail, the gills themselves, or the neighboring skin had no effect on them. When the tail of the control was touched it moved rapidly to the other end of the tank, but the operated animal only moved the tail slightly. Temperature of water 8.8°C.

March 18. The control had got out of the tank and was found lying on the floor, the gills retracted and motionless, at 8:00 a.m. It was returned to the tank, and began to move the gills vigorously (36 a minute at 9:32 a.m.). The operated animal was not moving the gills. Electrical stimulation caused immediate movements of the tail and legs. The left gills also began to move regularly (22.6 a minute). Electrical stimulation over the right gills, which were protruded

though motionless, caused them to be retracted at once. When laid on its side or back, the animal does not right itself. Water temperature 8.5°C.

March 21. Left gills moving but not very strongly. Right gills also moving slightly. Both are retracted when it is lifted out of the water, as in the case of the control. Returned to the water, it lifts the head up, and erects itself slightly on the forelimbs. Sometimes now the gills remain at rest on both sides, although protruded, for a few minutes. The animal moved a few inches when the control touched it. When held in the hand the head of the operated animal hangs down limp, but it makes a slight effort to turn on its belly if held on its side. In water, however, it lies on its back if placed in that position, although it has never been found on its back in the tank except once, on the morning after the operation.

March 22. Left gills still moving steadily; right protruded but motionless. The gills of the control are retracted and not moving.

March 23. The gills are moving on both sides regularly and pretty strongly. On being lifted from the water, it shut down the gills on both sides and kept them retracted and motionless, like the control, for the 5 minutes during which they were observed. They were not seen so long at rest since the operation. Twenty minutes later (in the tank) the gills on both sides were moving vigorously (34.3 a minute), although not always keeping time on the two sides.

March 25. Operated animal lying on its back and moving the gills strongly (63.2 a minute). The control was also moving the gills. The water had apparently been turned off, although it had been left running as usual 24 hours before.

March 28. The operated animal was found lying on the top of the control with both sets of gills contracting vigorously, and those of the control less strongly. Tried to feed the operated animal with a living tadpole. It opened its mouth widely but would not swallow. The tadpole was gently pushed down, but the Necturus resisted violently. Its body was contorted and the head moved far more strongly than at any time since the operation. On being thrown into the water it moved about very actively, threw up its head, opened its mouth, and turned over and over about its longitudinal axis. The strong contractions of the whole body were in marked contrast with the behavior of the animal since the operation.[2] In a few minutes the movements ceased and the animal lay in the usual position but with the anterior end of the body still elevated on the fore limbs, the mouth turned up, and the gills working fast, although not very regularly. The left gills began to move sooner than the right when it was put into the water. When it was taken out the gills stopped moving, but at once began again when it was replaced in the water, the left gills starting first. This was repeated in half an hour with the same result. The control also refused to swallow a tadpole and even vomited some fish already in the stomach, but although quite as much effort was made with forceps, etc., to get it to swallow, and although the pharynx was purposely irritated, it showed none of the movements of rotation of the operated animal when thrown into the water.

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[2] The strong and wide-spread muscular contractions, elicited apparently by stimulation of the oro-pharyngeal mucous membrane, are perhaps similar to the general and long-lasting convulsions sometimes observed in frogs, after removal of the cerebral hemispheres, on scratching the mucous membrane of the mouth with forceps (7). I have seen a decerebrate frog, which had been allowed to fall from a height of a few inches on its nose, immediately develop a violent general spasm, which lasted 5 minutes, the hind legs being strongly extended in line with the body and the anterior limbs strongly extended at right angles to the body.

March 29. The operated animal is lying with the gills fully extended but not in motion. The gills of the control are retracted and motionless. The wound is open.

March 30. The operated animal is on its back. Taken out of the water, it soon begins to move the head and tail quite strongly.

April 1. The operated animal is on its back. On pinching the toes of the hind leg it promptly pulls up the foot, and the toes of the contralateral foot twitch. The foreleg is also drawn up when the toes are pinched but there is no crossed reflex. The gills are at rest.

April 5. Gills fully protruded but not moving. Reflex movements of legs on pinching the toes. The control lies quietly with its gills retracted and motionless.

April 9. Demonstrated the operated animal to the class. It seemed in as good condition as at last observation, but next day it was dead, 4 weeks after the operation. The skin was much paler than normal.

Another animal, moved about in the water a few minutes after the operation (on April 16, 1895), would not lie on its back, made snapping movements with the jaws (these were seen and heard in a number of the animals) and ate frogs' eggs. The gills were fully expanded and moving regularly (36 a minute). On April 17th it was lying quietly with the gills moving regularly. On April 18th it was found lying on on its back, the gills moving at the rate of 52 a minute. It became quite lively when held in the hand, but did not eat. April 19th, the gills were retracted on stimulation, and did not move as regularly as before. No change till April 24th, when it is noted that the gills were moving, but more weakly. On April 26th and 28th the gills were moving, but not on April 27th at the time the animal was observed. On May 2nd, there was no change. The animal was quite lively when held in the hand. On May 3rd, the gills were not moving, and the wound was open. On May 5th the animal was dead (on the 19th day).

On February 28, 1896, the cerebral hemispheres of a Necturus were excised under ether. Immediately after the operation it would lie on its back when placed in that position in water, but made efforts to right itself, and with a little help could do so. Snapping of the jaws was observed during and for some time after the operation. It moved pretty freely when pinched. The gills were moving as usual after excision of the hemispheres. On March 9th it was laid on its back on a table and a pair of forceps put into its mouth. It snapped vigorously on the forceps and ultimately flung itself round on its belly, expelling the forceps at the same time. In the water it was always found on its belly, but did not readily turn when placed on its back. On March 23rd it was found dead.

The capacity of recovering the normal position when placed on the back immediately after the operation may depend in some measure on the degree of anesthesia. Thus Necturus G righted itself immediately after the operation (on March 19, 1896). There were incessant move-

ments of the gills. On the following day the condition was the same. A week thereafter it still recovered the normal position but with difficulty. It sank to the bottom of the tank and lay there for a little time on its back before turning. On the fourteenth day it was found dead.

Lack of coördination in the gill movements on the two sides has occasionally been observed. Thus, after removal of the cerebral hemispheres from a very large specimen (Necturus F) incessant rhythmical contractions of the gills, perfectly coördinated on the two sides, were noticed as soon as it was put into the water after the operation. It did not quite succeed in righting itself when placed on its back, although it made vigorous efforts to do so. Next day the righting reaction was prompt and complete, but there was now some lack of coördination of the gill movements, the left moving more frequently than the right gills. It is not known whether this condition was due to further progressive damage after the operation. But this is not always the explanation. For in at least one instance it was seen that the incoördination, which developed some time after the operation, disappeared later on.

In one animal practically the whole brain in front of the anterior border of the medulla oblongata was removed on March 13, 1896. The gills were moving rhythmically immediately after the operation, and continued to do so. On March 24th they were not moving when observations were made, and on March 27th the animal was found dead. At no time did it make any effort to right itself when placed on its back, and was usually found lying on its back in the water.

In an animal from which the brain was removed in front of a transverse line a little anterior to the anterior margin of the otic capsules, rhythmical movements of the gills began almost as soon as the animal was put into the water. These were always seen when observations were made during the first two days, but not later on. The animal only lived 4 days. At any time after the operation the animal would lie on its back in water, and although it would remain in the natural position when placed in it, it was also sometimes found on its back in the tank. The head and anterior part of the body were apt to be erected on the forefeet. During the operation a snapping of the jaws was seen and heard when a small sponge was put into the brain cavity. It continued to snap at intervals after being put into the water on the evening after the operation, but this was not observed on succeeding days.

The effect on the gill movements of destruction of the fore-brain suggests that an inhibition has been removed which is normally concerned in their regulation, and that these movements, while related closely to the nervous system, do not depend essentially upon the condition of the blood. For the intensity of metabolism must probably be decidedly diminished in the decerebrate animal, which lies

for long periods without changing its position, as compared with the normal animal. Babak (5) also concludes that diminution in the oxygen supply has no noticeable influence upon the gill movements. The usual effect of reflex stimulation was an inhibition of the movements both in the normal and in the operated animals, although the inhibition was transient. Obviously the inhibition can be produced through some mechanism posterior to the cerebral hemispheres. Babak has observed reflex inhibition in the normal animal. We have seen also the initiation of movements caused by stimulation in the operated animal if the gills were at rest.

The gill movements may sometimes be associated with spontaneous movements of the normal animal or movements following stimulation of the decerebrate animal. But there is nothing to suggest that the rhythmical discharge of the center controlling them is essentially dependent upon reflex afferent impulses.

The contact of water with the skin or with the gills themselves seems to constitute an important condition of their activity. For they stop moving and are retracted when the animal is taken from the water and kept in the air. If protruded but not moving, they are retracted, although in the case of the operated animals not perhaps always completely. Either then the contact or the resistance of the water supplies some necessary stimulus, or the sudden change from water to air causes an inhibitory excitation. Babak (5) states that he has seen gill movements in the air in the two (normal) specimens studied by him. This was certainly rare in our experience. It must not be forgotten that the gills, during the movements of which the richly vascular tufts are alternately emptied and filled with blood, constitute not only a respiratory mechanism, but in some degree perhaps an accessory circulatory mechanism. That the gills are not continuously in motion is no doubt explained by the fact that the animal has other respiratory organs (the skin, lungs and possibly to some extent the oro-pharyngeal walls).

Whether the gill movements are discharged "automatically" after the removal of a detent in the form of a central inhibition, as the effect of removal of the cerebral hemispheres might suggest, or in response to peripheral reflex impulses which lower the threshold of the center, cannot be decided from the observations made. It may be again pointed out, however, that the usual effect of reflex stimulation is to inhibit the movements. Kuiper (8) has discussed the question of respiratory automatism in connection with the respiration of teleostean fishes. He concludes that in all classes of vertebrates the respiratory centers act automatically, that is, continue to send out rhythmical impulses for the respiratory movements after interruption of all afferent nervous paths. In mammals, one of the best methods of isolating the

respiratory center from afferent impulses is temporary anemia of the brain, the bulb and the anterior segments of the cervical cord, produced by ligation of the head arteries (9). At a certain stage in the anemia and again at a certain stage in recovery after release of the vessels the respiratory center can be seen to be discharging rhythmical impulses, when to all tests that can be applied all possible afferent paths are closed. At this stage, section of the brain stem with the knife above the level of the respiratory center, or of the vagi, has no effect on the respiratory discharge, any more than stimulation of the central ends of nerves like the brachial or of the vagus. Effective impulses coming normally to the center from the lungs along the vagi, or from parts of the brain or along cranial nerves above the level of the section cannot be reaching the center at this time when impulses set up by artificial stimulation, however strong, are ineffective. Division of the cord in the lower cervical region and elimination of the whole of the lower part of the cord by ligation of the thoracic aorta, in addition to ligation of the head arteries also do not essentially affect the return of respiration after temporary anemia of the brain. It is therefore impossible to believe, with Baglioni (6), that the respiratory discharge under these conditions is due to reflexes connected with the position of the thorax, especially as there is no evidence at all that such reflex impulses, if normally generated, can pass through an anemic area quite impermeable to the strongest artificially excited impulses. As regards his idea that reflex impulses from the diaphragm may be responsible for the rhythmical discharge of the respiratory center isolated by temporary anemia, it is sufficient to say that the diaphragm can be eliminated without interfering essentially with the respiratory discharge in resuscitation after a period of cerebral anemia, as seen, for example, in the respiratory movements of the head of an animal engrafted on the circulation of another animal (10), (11).

BIBLIOGRAPHY

(1) Carpenter: Principles of comparative physiology. (Amer. ed. from the 4th English ed.) Philadelphia, 1854, 326.
(2) Longet: Traité de physiologie, Paris, 1852, i, 456.
(3) Stewart: Manual of physiology, 1st ed., 1895, 655; 8th ed., 1918, 947.
(4) Gadow: The Cambridge natural history, 1909, viii, 132.
(5) Babak: Arch. f. d. gesammt. Physiol., 1913, cliii, 441.
(6) Baglioni: Ergebn. d. Physiol., 1911, 526.
(7) Stewart: The American year book of medicine and surgery, Philadelphia, 1898, 988.
(8) Kuiper: Arch. f. d. gesammt. Physiol., 1907, cxvii, 1.
(9) Stewart and Pike: This Journal, 1907, xix, 328; Ibid., 1907, xx, 61.
(10) Guthrie, Pike and Stewart: This Journal, 1906, xvii, 344.
(11) Guthrie: Bloodvessel surgery, 1912, 118, 250.
(12) Luckhardt and Carlson: This Journal, 1920, liv, 122.
(13) Stewart: Arch. néerland. de physiol., 1922, vii, 340.

THE QUANTITATIVE DETERMINATION OF TRYPSIN AND LIPASE IN GASTRIC CONTENTS[1,2]

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The regurgitation of pancreatic juice through the duodenal orifice into the stomach is a very common phenomenon in digestion, the significance of which is as yet only partially understood. It is known that when high acidities develop in the stomach, or are induced by the ingestion of considerable amounts of acid, regurgitation of pancreatic juice and bile commonly follow, the acidity being thus reduced and evacuation promoted. This process likewise occurs when the gastric contents are subacid or even alkaline in reaction. It appears also during starvation. This reverse movement through the pylorus begins normally toward the close of the digestive phase. The time of evacuation of gastric contents is in a measure dependent upon an initial regurgitation of alkaline duodenal juice, which by lowering the acidity of the chyme, effects a more prompt relaxation of the pyloric sphincter, and assures a non-irritating action upon the duodenal mucosa, (1). It is known also that fatty foods promote regurgitation of pancreatic juice and bile into the stomach. Theories have been advanced in explanation of this phenomenon (2). Regurgitation thus appears to be a regulatory mechanism in digestion (3) interference with which from the standpoint of excess or deficiency may be of clinical significance.

It is of interest here to mention the viewpoint of Alvarez (4), who explains the regurgitation toward the end of digestion by his gradient theory and assumes that "the tone of the stomach drops below that of the digesting bowel." Following the direct injection of barium into the human duodenum, radiographic observations made by Wheelon (5) seem to support the gradient theory. The same author also concluded that the mechanics of the duodenum are responsible for the retention of barium in the inferior flexure, the cap and the antrum:

[1] Read before the Section on Internal Medicine, College of Physicians, Philadelphia, March 26, 1923.

[2] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson.

"The average duodenum has its lowest point at the inferior flexure; the portion reaching the duodeno-jejunal flexure rises rather acutely. . . . . Material must be forcibly lifted against gravity from the lowest point."

One of us (6) in gastric studies made upon fifty-eight patients, most of them being mental patients in the psychopathic wards of the Pennsylvania Hospital, Philadelphia, found regurgitation present in fifty-nine of the ninty-one fractional studies made. Pancreatic trypsin and lipase were recovered quantitatively in thirty-eight of forty-two patients of the mental group or in 90 per cent. Thirty-two fasting stomach contents in the same group contained the above ferments, or 76 per cent. In thirty, the ferments appeared toward the close of digestion, or in 71 per cent; the ferments were found throughout digestion in but fourteen patients or in 33 per cent. This last division included patients with subacidity, anacidity and achylia, with but few exceptions. To these authors, regurgitation represents possibly a compensatory factor in gastric digestion, through its enzyme content and bile, in addition to its protective and regulatory function.

As measures of pancreatic regurgitation trypsin, because of its absence from the salivary and gastric secretions, and lipase, because it is found only in small amounts in gastric juice, are the most available. The casein method of Spencer (7) for the determination of trypsin gives very satisfactory results for small amounts of this enzyme. A simple method is, however, much to be desired where a large number of specimens is to be examined as in the fractional method of gastric analysis or investigations of similar type.

We desire, therefore, to suggest two simple methods for the determination of trypsin and lipase respectively in gastric contents. The principles of these methods are not new, but we believe they have not previously been applied to gastric contents. They follow closely the methods recently suggested from this laboratory for the determination of trypsin and lipase in duodenal contents (8). Amylase may be determined in gastric contents also by the method described in the same connection. Amylase may, however, also arise from swallowed saliva.

Methods. *Collection of samples.* First remove completely the fasting contents. Fifty cubic centimeters is considered the average normal amount. This initial emptying is done in order to learn if any, and at what time during digestion, duodenal regurgitation takes place. It has occasionally been our experience that a fasting content, retained except for a 5 cc. withdrawal rich in bile and ferments, caused the presence of bile and ferments in every 15 minute sample, throughout gastric digestion. Later tests, with complete removal of fasting contents, showed bile and pancreatic ferments in fasting contents and

toward end of digestive phase. Completely empty stomach at end of third hour, allow patient to drink 100 cc. of water, and immediately remove this lavage to learn if complete evacuation of fluid and food has taken place. Keep each 5 cc. sample in tubes, in beaker containing cracked ice, to prevent deterioration of enzymes. Determine the enzyme activity as soon as possible after collection of samples.

The methods suggested for trypsin and lipase are as follows:

Procedure for the determination of trypsin. *a.* Prepare as many 50 cc. Erlenmeyer flasks as the number of specimens of gastric contents collected for fractional study. Number flasks T-1, T-2, T-3, etc.

b. To each flask add 10 cc. of 2.5 per cent gelatin[3] and one drop of phenolphthalein (1 per cent alcoholic solution). Then add, drop by drop, N/10 NaOH until a light pink color is produced.

c. To each flask add, by means of an Ostwald pipette, 1 cc. of strained gastric contents, and again titrate to a light pink color, preferably with N/100 NaOH.

d. Incubate for one hour at 37°C. At the same time incubate a flask (control) containing 10 cc. of gelatin solution made neutral as above. Shake flasks every 15 minutes.

e. During the digestion test, determine the amount of amino acid nitrogen by measuring out 1 cc. of gastric contents in a small porcelain dish, adding one drop of phenolphthalein solution, titrating with N/100 NaOH to a light pink color, then adding 0.5 cc. of neutral formol-alcohol solution[4] and again titrating to the same tint.

f. Upon removal from the incubator place the flasks in ice water, to stop digestion.

g. Add to each flask 1 cc. of neutral formol-alcohol solution. Titrate to neutrality with N/100 NaOH. Take burette reading. Subtract blank *d*. Subtract the amino-acid nitrogen of 1 cc. gastric contents *e*. This figure, multiplied by 10, represents the number of cubic centimeters of N/10 NaOH necessary to neutralize the amino acids, etc., formed by the action of 100 cc. of gastric contents.

[3] To prepare a 2.5 per cent solution of gelatin, weigh out 25 grams of highest grade culture media gelatin and add it to a liter of distilled water in a large beaker over a bunsen burner. The temperature of the water should be kept below 60°C., to prevent scorching of the gelatin. Stir continuously until solution becomes clear and homogeneous. Pour carefully into a clean, previously heated bottle (to prevent cracking) and add toluene to cover solution with ¼ inch layer. Keep at room temperature.

[4] To prepare formol-alcohol solution, take equal parts of a 40 per cent formaldehyde solution and alcohol (95 per cent), added together to make a liter. Add 5 drops of phenolphthalein (1 per cent alcoholic solution) and concentrated NaOH until first light pink color persists on thorough shaking. Keep at room temperature.

Procedure for the determination of lipase. *a.* Prepare as many 25 cc. Erlenmeyer flasks as the number of specimens of gastric contents collected for fractional study. Number flasks L-1, L-2, L-3, etc.

b. To each flask add 5 cc. of a 20 per cent emulsion of olive oil[5] and one drop of phenolphthalein (1 per cent alcoholic solution). Then add, drop by drop, N/10 NaOH until a light pink color is produced.

c. To each flask add, by means of an Ostwald pipette, 1 cc. of gastric contents (strained through cheese cloth if necessary) and again titrate to a light pink color, using first N/10 and finally N/100 NaOH.

d. Incubate, for one hour, at 37°C. At the same time incubate a flask (marked control), containing 5 cc. of olive oil emulsion and 1 cc. of boiled gastric contents made neutral as above. Shake all flasks every 15 minutes. Upon removal from the incubator place flasks in ice water, to stop digestion.

e. Add 5 cc. of neutral alcohol-ether solution.[6] Titrate with N/100 NaOH to the first light pink color. Take burette reading. Subtract blank reading. The result, multiplied by 10, is a measure of the fatty acids derived from the lipase activity of 100 cc. of gastric contents expressed in terms of $\frac{N}{10}$ acidity. Where, for any reason, the lipase figures are expected to be high, neutralize digestion mixture with N/10 NaOH instead of with N/100, then multiply by 100 for the final results.

Discussion. Figures 1 to 3 show the availability of these methods in gastric studies and the character of the results which may be obtained with their aid. Figure 1 is particularly instructive as showing how we can follow fluctuations in enzyme concentration after the ingestion of a fatty food. The repeated regurgitation, the inverse character of acid and pancreatic enzyme curves, and the increasingly pancreatic character of the gastric digestion of fats is well shown. Figure 2 also shows how completely pancreatic in character gastric digestion may become and to what a considerable extent fats may be digested in the stomach. It is probable that some tryptic destruction of lipase has occurred in

[5] A 20 per cent emulsion of olive oil. Have at hand 30 grams of gum acacia, 60 cc. of distilled water, and 120 cc. of finest quality olive oil. Place the acacia in a mortar, add the oil and rub into a smooth paste, always stirring in one direction. When a thick homogeneous paste results, add, all at once, the 60 cc. of distilled water and continue stirring until a milk-white emulsion is formed. Measure into a graduated cylinder, and add distilled water with repeated washing of the mortar until 600 cc. of emulsion are made. Add 1 c.c. of 40 per cent formaldehyde, as a preservative. If a yellowish-tinged emulsion results, repeat the procedure.

[6] Alcohol-ether solution. Five parts of neutral alcohol (95 per cent) and one part of acid-free ether are mixed fresh for each day. Make mixture neutral with N/10 NaOH.

the low acidities. It is very important that determinations of pancreatic enzymes in gastric contents be made immediately after withdrawal. We desire to thank Dr. R. A. Lichtenthaeler for carrying out the acidity titrations in these cases.

The determinations charted in figure 3 were made on a subject who was given no food at all; the gastric stimulation in this case being purely psychic. Even here it will be noted that after a preliminary secretion of gastric juice the contents of the stomach may become quite completely pancreatic in character.

CONCLUSIONS

Simple methods are described for the determination of trypsin and lipase in gastric contents. These are capable of throwing considerable light upon the phenomenon of pancreatic regurgitation, which we believe to be of greater importance than generally realized.

BIBLIOGRAPHY

(1) Spencer, Meyer, Rehfuss and Hawk: This Journal, 1916, xxxix, 459.
(2) Bokai: Arch. f. exper. Path. u. Pharm., 1887, xxiv, 153.
Cannon: This Journal, 1907, xx, 283.
(3) Boldyreff: Quart. Journ. Exper. Physiol., 1914, viii, 1.
(4) Alvarez: The mechanics of the digestive tract, New York, 1922.
(5) Wheelon: Journ. Amer. Med. Assoc., 1923, lxxx, 615.
(6) Farr and Lueders: Gastric secretory function in the psychoses." Read before the American Gastro-Enterological Society, Atlantic City, N. J., May 1, 1923.
(7) Spencer: Journ. Biol. Chem., 1915, xxi, 165.
(8) Lueders, Bergeim and Rehfuss: Amer. Journ. Med. Sci., in press.

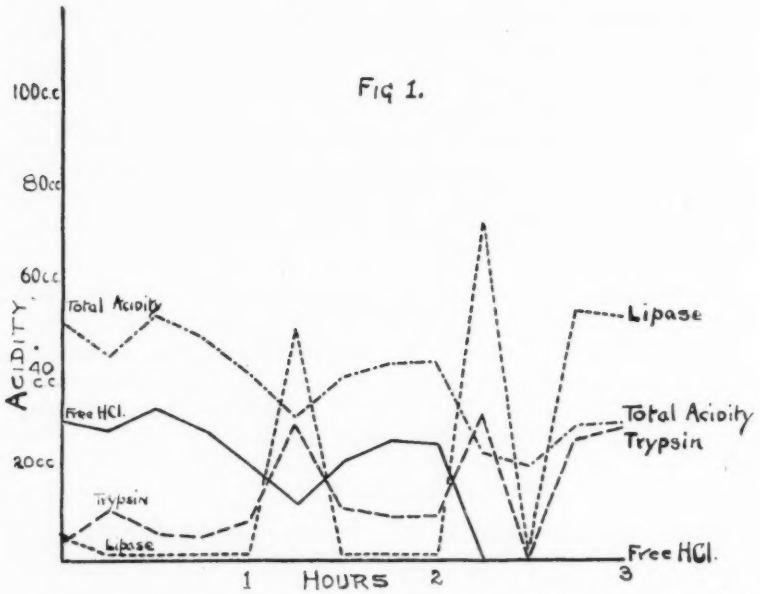

Fig. 1. Acid and enzyme values for gastric contents following ingestion of 20 grams of butter.

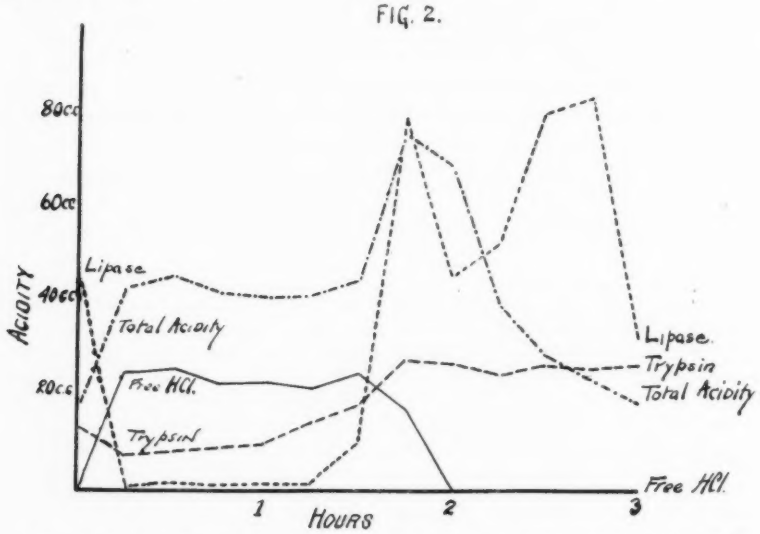

Fig. 2. Acid and enzyme values for gastric contents following ingestion of 20 grams of butter.

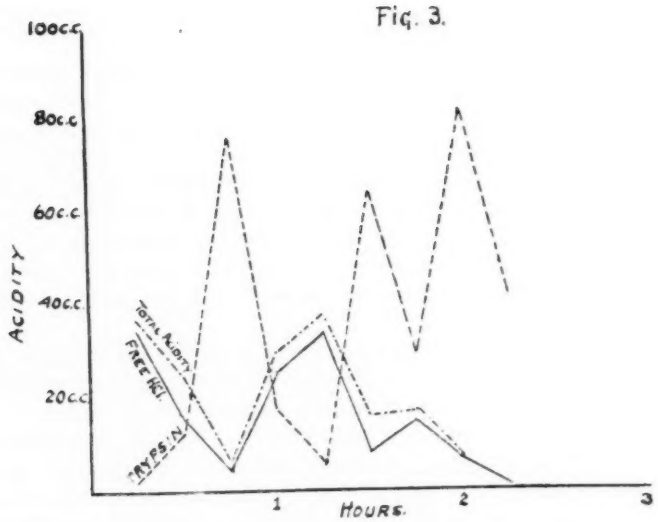

Fig. 3. Acid and enzyme values for gastric contents following psychic stimulation.

THE EFFECT OF ASPHYXIA UPON THE ADRENAL OUTPUT AS DEMONSTRATED BY VARIATIONS IN THE TEMPERATURE OF THE BRAIN

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In view of the findings by Cannon (1), (2) and by other investigators that the secretion of epinephrin is increased by asphyxia, an observation which had also been made by one of us in a previous research; and since it had been demonstrated in the bio-physical laboratories of the Cleveland Clinic that the injection of adrenalin in normal animals produces a typical increase in the temperature of the brain, it seemed that it would be of interest to measure the temperature of the brain during asphyxia, both in animals with intact adrenals and in animals from which the adrenals had been removed. A series of eleven experiments was performed (table 1).

In two normal animals the temperature of the brain during the period of asphyxia rose 0.51°C., and 0.98°C. respectively. Subsequent periods of asphyxia in these animals were also accompanied by a rise in brain temperature to a lesser degree (fig. 1). One animal was heavily narcotized with morphin. Asphyxia while the animal was deeply narcotized caused an increase in the temperature of the brain of only 0.04°C. Three hours later, however, when the period of narcotization was nearly passed the temperature of the brain during the period of asphyxia rose 0.26°C. (fig. 2). In marked contrast to the effect of asphyxia in animals narcotized by morphin was the effect in animals which had received a preliminary dose of strychnin. In each of two animals which had received $\frac{1}{75}$ of a grain of strychnin five minutes before each was subjected to asphyxia, the temperature of the brain rose 0.4°C. In three animals the adrenals were removed, the operation being completed ten minutes before the period of asphyxia. In each of these animals the temperature curve of the brain was unaltered by the asphyxia. The injection of adrenalin in each of these caused an abrupt and large increase in the temperature of the brain of from 1 to 2.09°C. (fig. 3). Since in each of these last three animals

[1]Read before the American Physiological Society, Toronto, December 27-29, 1922.

TABLE I

The effect of asphyxia upon the output of epinephrin as indicated by variations in the temperature of the brain

EXPERIMENT	WEIGHT OF RABBIT	PRELIMINARY PROCEDURE	PERIOD OF ASPHYXIA I	RISE IN TEMPERATURE OF BRAIN	PERIOD OF ASPHYXIA II	RISE IN TEMPERATURE OF BRAIN	PERIOD OF ASPHYXIA III	RISE IN TEMPERATURE OF BRAIN	RISE OF BRAIN TEMPERATURE AFTER INJECTION OF ADRENALIN
	lbs.		*min.*	*°C.*		*°C.*		*°C.*	
1. Normal	6		5	+0 51	4½ min.	+0 19	3½ min.	+0 13	+0 67°C.
2. Normal	4½		7½	+0 98	7½ min.	+0 84			
3. Morphin	6	Morph. sulph. gr. 2½ in 2 doses—Gr. 2, 1hr and Gr. ½, −½hr. before first asphyxia	5½	+0 04	(3 hrs. 39 min. after morphin dose 2) 4½ min.	+0 26	(3 hrs. 53 min. after morphin dose 2) 4 min.	+0 21	Dose I 5 min. after asphyxia I +0 29°C. dose 2 immediately after asphyxia III +0 25°C.
4. Strychnin	6	Strych. sulph. gr. $\frac{1}{15}$, 5½ min. before asphyxia	5	+0 42					
5. Strychnin	5	Strych. sulph. gr. $\frac{1}{15}$, 4 min. before asphyxia	3½	+0 4					
6. Adrenalectomy	5½	Double adrenalectomy under ether anesthesia 9 min. before asphyxia	3½	*Fall* −0 28					+2 09°C.
7. Adrenalectomy	5½	Double adrenalectomy under ether anesthesia 11 min. before asphyxia	4	*Fall* −0 18					1 52°C.
8. Adrenalectomy	6¼	Double adrenalectomy under ether anesthesia 10 min. before asphyxia	2½	*Fall* −0 12	1 min.	*Fall* −0 09			+1 0°C.
9. Abdominal shock	5¾	Abd. manip. and exposure approximately equiv. to that of removing adrenals 13 min. before asphyxia	11	+0 64	7 min.	+0 44			

TABLE 1—*Concluded*

EXPERIMENT	WEIGHT OF RABBIT	PRELIMINARY PROCEDURE	PERIOD OF ASPHYXIA I	RISE IN TEMPERATURE OF BRAIN	PERIOD OF ASPHYXIA II	RISE IN TEMPERATURE OF BRAIN	PERIOD OF ASPHYXIA III	RISE IN TEMPERATURE OF BRAIN	RISE OF BRAIN TEMPERATURE AFTER INJECTION OF ADRENALIN
	lbs.		*min.*	°*C.*		°*C.*		°*C.*	
10. Abdominal shock		Abd. manip. and exposure approximately equiv. to that of removing adrenals 12 min. before asphyxia	4	+0 36	2 min.	+0 27	4 min.	0 2	
11. Abdominal shock partial adrenalectomy		(See note) Operation completed 11 min. before asphyxia	5	+0 37	7½ min.	+0 47			

Note. This was supposed to be a complete double adrenalectomy; the rise in temperature accompanying asphyxia led to [illegible] which showed one-third of right adrenal *in situ*.

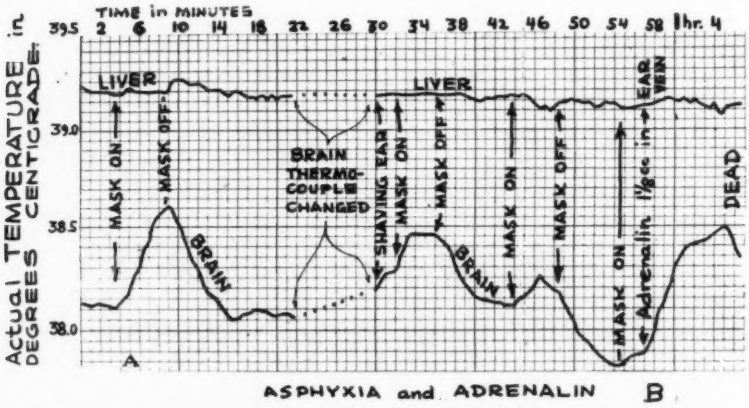

Fig. 1. Effect of asphyxia upon the temperature of the brain. Compare the response of the brain to asphyxia alone, *A*, with the response to an injection of adrenalin, *B*.

the shock of the operation was necessarily great and the temperature of the brain was falling steadily, it was suggested that the lack of response to the asphyxia might be due to the degree of shock in each animal. In two animals, therefore, under ether anesthesia for the same period as that required for adrenalectomy, the abdomen was opened and the intestines were exposed and manipulated. At the conclusion

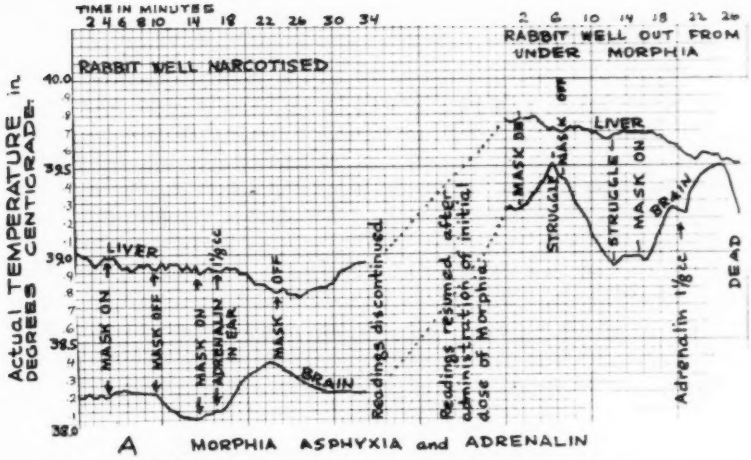

Fig. 2. Effect of asphyxia upon the temperature of the brain in a morphinized animal. Compare the lack of response at *A* with *A* in figure 1.

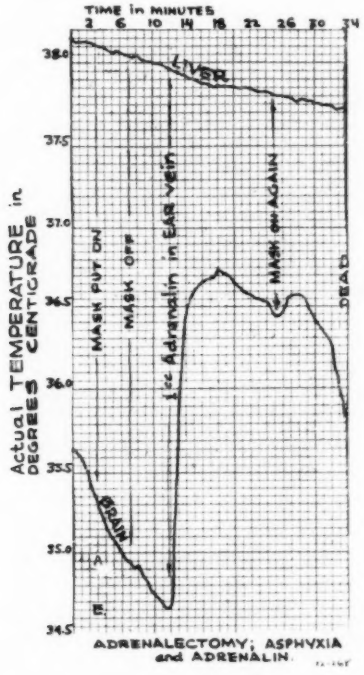

Fig. 3. Effect of asphyxia upon the temperature of the brain in an animal from which the adrenals had been removed. Note the enormous response to the injection of adrenalin as compared with the complete lack of response during the period of asphyxia, (A).

of the manipulation the temperature of the brain of each of these animals was falling at approximately the same rate as in animals in which the adrenals had been removed; but in the two animals subjected to shock alone during asphyxia the temperature of the brain rose 0.64°C. and 0.36°C. respectively (fig. 4). In another animal in which an attempt was made to perform a complete double adrenalectomy the temperature of the brain rose 0.37°C. during asphyxia. Autopsy, however, showed that one-third of the right adrenal was *in situ*. In none of these experiments was any alteration in the temperature of the liver observed, excepting in the animals which had received a preliminary dose of strychnin in which the temperature of the liver fell during the period of asphyxia.

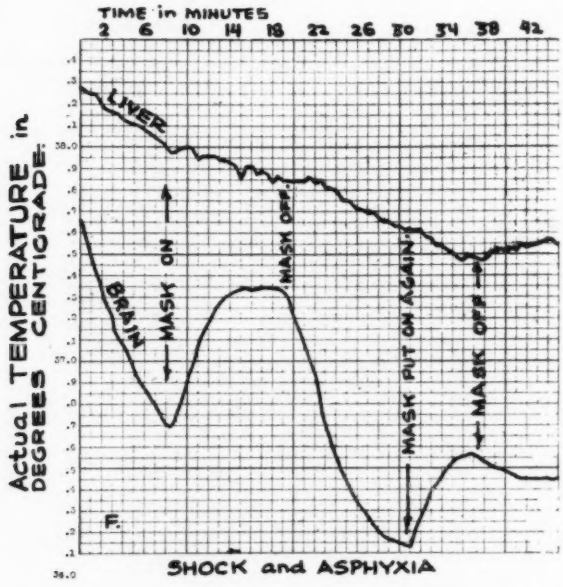

Fig. 4. Effect of asphyxia upon the temperature of the brain in an animal in shock.

CONCLUSION

Asphyxia produces an increased output of epinephrin which is manifested by an increase in the temperature of the brain.

BIBLIOGRAPHY

(1) CANNON AND HOSKINS: This Journal, 1911, xxix, 275.
(2) CANNON: Bodily changes in pain, hunger, fear and rage, 1915, 206.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XIII. Asphyxial Death of Embryos in Eggs Abnormally Retained in the Oviduct

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Various students of poultry have observed that an occasional fresh-laid egg of the common fowl already contains a live or dead embryo of one, two or three days of growth. In these cases it is obvious that such eggs were retained or delayed within the hen's oviduct during at least the period represented by the age of the embryo. Why some of these embryos are dead and others alive is a question which seems never to have been investigated. That the retention of an egg in the oviduct may cause sickness or death in the parent bird is also well known; but we have no information as to how frequently this observed and unobserved delay actually occurs. Reproductive habits and conditions in the fowl are unfavorable for a determination of these points, but specially favorable conditions are found in the pigeon.

In our own studies, and in all genetic work with birds, it may be of importance to know the cause of failure or death in all eggs or embryos which fail or die. For example, in some pigeons the two eggs of the pair or clutch have unequal prospective sex values and in any complete analysis of sex ratios in these forms it is necessary to know whether the first or the second of the clutch is more subject to delay and consequent death. The causes of embryonic or fetal death are also a necessary and important part of the physiology of animal reproduction. The full purpose of the present paper is to show that we are now able to recognize that the retention of eggs in the oviduct is a source of embryonic death; to point out the factor immediately responsible for the death; to measure the amount of death from this cause; and to evaluate the extent to which these eliminations by death may affect sex and genetic ratios obtained in pigeon breeding.

In this study we have been able to obtain three kinds of fairly accurate data upon 50 fertile eggs retained in the oviduct from 5 to 122 hours

beyond the normal period. An inspection of these data will show that death or survival of the enclosed embryos is decided on the basis of two factors—the duration of the delay within the oviduct, and the thickness of the shells formed on the eggs (the shell thickness measured by a determination of the rate of loss of water vapor from the egg during subsequent incubation). In other work done in this laboratory the oxygen requirements of the dove embryo in the various post-laying stages of development have been measured. The present results on death or survival in the retained eggs—death in thick shells and survival in thin shells—combined with the known results on oxygen requirements, lead to the conclusion that death in these retained eggs is the result of an inadequate oxygen supply. The thicker the shell the more restricted is the oxygen supply to the embryo; and the more advanced the embryonic stage the greater is the absolute oxygen requirement. The embryos in retained thick-shelled eggs therefore die at an early stage. The embryonic death resulting from oviducal retention probably tends selectively to eliminate more females than males in eggs from pure species of doves and pigeons; but in our own pens this elimination probably does not lower the proportion of females by more than 0.3 of 1 per cent. About 0.5 to 0.6 per cent of all dove embryos are killed by retention of the egg in the oviduct.

PRESENTATION OF DATA. *Data obtained from 50 fertile eggs retained in the oviduct of the pigeon.* The data are divided into three parts in table 1. Within each part the eggs are listed in the order of amount of delay—the longest periods of delay being given first. The shell thickness as measured by the rate of loss of water from the egg is given in the third column of figures. The justification of this method of measurement has been given by us elsewhere (1). Since this rate of loss is also related to the amount of shell surface, and this varies in eggs of different size, the egg weight is here recorded as a sufficient index of surface. Some special aspects of the data for rate of loss will be considered below.

Two columns of data give the duration of the retention of the eggs in the oviduct. In the first column the amount definitely known; and in the second, the most probable amount of the delay. It will assist the reader to understand the definiteness of the figures given for the amount of delay if we give here a short statement concerning the time relations of ovulation and egg-laying in pigeons as these facts have been learned by ourselves and by others. The egg of the common pigeon requires about 42 hours to pass from ovary to exterior; in the ring doves this is accomplished in about 38 hours. *The time of egg-laying is narrowly fixed in pigeons.* Ring doves lay the first of the clutch at close to 5:00 p.m.; the second, at 9:00 a.m., 40 hours later.

TABLE 1

Details concerning death or survival of embryos found in eggs abnormally retained in the oviduct

CASE NUMBER	DATA ON EGGS				DATA ON EMBRYO	
	Weight (grams)	Rate of loss (mgm. per hour)	Amount of delay (in hours)		Percentage of 10 adjacent eggs developed to 2+ days	Stage found after further incubation (all dead except as noted)
			Certain	Probable		
Ring doves						
1	8.2	0.9	122			0.5-day
2	8.0		80	97	90	0.5-day
3	7.0	0.9	81	83	100	0.5-day
4	7.5	1.1	68		90	0.5-day
5	6.9		43	67	100	1.0-day
6	8.6	2.0	57	65	100	0.8-day
7	7.1	4.4	54	60	90	1.0-day
8	8.6	3.8	48	60	90	0.5-day
9	8.9	3.2	45*	52	100	3-day embryo
10	7.7	1.1	52		100	0.8-day embryo
11	8.0	6.5	48		100	8-day embryo
12	8.1	2.0	48		100	0.8-day
13	6.3	0.1	34		70	0.5-day
14	7.8	(8.5)**	5	29	100	4-5 days, killed
15	9.6	7.5	11	28	90	0.5-day
16	8.3	1.8	26		100	0.8-day
17	8.6	4.1	25		100	Hatched
18	8.5	5.1	25†		100	4-day embryo
19	8.0	2.0	24	25	100	Hatched
20	8.6	2.7	24		70	Hatched
21	8.0	2.9	24		80	4.5-days
22	8.7	0.9	23		100	0.5-day
23	9.2	1.2	10	11	90	0.5-day
24	9.3	3.4	3	6–7	100	Hatched
25	8.6	3.8	3	4–6	100	Hatched
An abnormal ring dove						
26	6.2	1.7	72			0.5-day
27	7.0	2.0	65	67		Fertile (?)
28	7.6	2.7	53	60		0.8-day
29	6.4	1.1	53	60		0.5-day
30	7.4	1.2	48	60		0.5-day
31	6.2	4.5	52	53		0.5-day
32	7.8	1.7	50	51	50§	0.5-day
33	7.7		50			Fertile, broken
34	7.0	5.9	49			0.8-day
35	7.5	18.2	25*	26		0.5-day
36	7.1	(10.8)	6	12		2-day embryo
37	4.8	(11.5)	2	12		0.5-day
38	6.8	4.1	2	5–14		0.5-day

TABLE 1—*Concluded*

CASE NUMBER	DATA ON EGGS				DATA ON EMBRYO	
	Weight (grams)	Rate of loss (mgm. per hour)	Amount of delay (in hours)		Percentage of 10 adjacent eggs developed to 2+ days	Stage found after further incubation (all dead except as noted)
			Certain	Probable		
Common pigeons						
39	12.0	1.1	119	120	90	0.5-day
40	14.9	0.9	44	48	90	0.5-day
41	16.6	3.0	23	46	100	0.8-day
42	15.3	1.9	31	36	70	0.5-day
43	12.4	2.2	22	24	80	3.0-day embryo
44	18.1	8.2	22*	24	70	Hatched
45	18.3	5.1	20	24	100	0.8-day
46	17.4		21*	24	100	0.5-day
47	16.2	10.4	21*	24	100	Hatched
48	16.1	4.9	24*		90	17.0-day embryo
49	20.5		20*	24	90	Hatched
50	18.4	6.5	12*		100	Hatched

* Shell remained soft to within 24 hours of time actually laid. For common pigeons this mark further indicates the delay probably occurred in the ovary

** Figures in parentheses in this column had plainly inadequate or (at last weighing) slightly crushed shells.

‡ Possibly this egg was delayed in the ovary, not in the oviduct.

§ This figure is for all eggs produced between the first and last delayed eggs (3/26–8/12). The delayed eggs listed here and the soft-shelled eggs incapable of external incubation are not included. All of this group of eggs were from the same parent.

Common pigeons lay their eggs between 2:00 p.m. and 4:00 p.m.—the first of the pair nearer the later hour, and the second nearer the earlier hour. In the case of both groups the oviducal egg can be palpated with the finger placed between the bird's pubic bones at 30 to 33 hours before it is to be laid. At any time up to within 24 hours of laying—in either normal or retained eggs—an experienced observer can also readily learn whether it is a firm shell or a soft shell that is then present on the palpated egg. By this means cases 9, 18, 35, 46, 47, 48, 49, of our table were known to have been soft-shelled at about 24 hours before they were actually laid. The whole of our information on the four last-named cases makes it probable that the indicated delay occurred in the ovary, not in the oviduct.

It is further notable in connection with the above facts that a long retention within the shell-gland is usually but not necessarily accompanied by a heavy deposit of shell substance. Cases 7, 8, 9, 11, 14, 15, 17, 18, 20, 28, 31, 34 and 35 are all exceptions. Their shells were

either of nearly normal thickness—as shown by the figures in the rate of loss column—or they were too thin, though all these eggs were retained an extra twenty-four or more hours in the shell gland.

At this point we may more definitely indicate what is meant by a shell of normal thickness—as measured by the rate at which the egg loses weight. For this purpose we can use the data of an earlier publication (1, tables 2, 3). The eggs of three ring-doves yielding "normal" shells gave average rates of loss of 3.8, 3.9 and 4.2 mgm. per hour. The total range of variation in 24 eggs from these three birds was from 2.6 to 6.3 mgm. per hour, on eggs whose weight varied between 6.5 and 10.1 grams. As pointed out in that paper, a rate of loss of more than 5.0 mgm. from an egg of 9.0 grams or less indicates an inadequate shell. In that earlier study six birds known to produce some eggs with inadequate shells yielded the following averages for water loss: 4.6, 4.8, 5.3, 6.0, 6.7 and 7.5. The rates of water loss from the thirty-one eggs varied from 2.6 to 10.5 mgm. per hour for eggs whose weights ranged from 6.9 to 10.0 grams. The normal rate of loss for ring dove eggs of 7.0 to 10.0 grams certainly lies between 2.6 and 5.0 mgm. per hour. For common pigeon eggs weighing between 12.0 to 20.0 grams this rate is certainly between 5.0 and 10.0 mgm. per hour. This statement is based upon many hundreds of measurements which it seems unnecessary to give here. Shell measurements were unfortunately omitted in five cases of the present series; this occurred at the beginning of the study and in one case of breakage and in one case of neglect.

In the next column of the table data are given for the developmental capacities of other eggs produced by the birds which yielded the retained eggs. It will be seen that nearly all of the unretained eggs being produced by these birds were capable of producing embryos which lived at least to stages older than 2.0 days; in contrast, two-thirds of the embryos in the retained eggs—excepting the seven eggs retained less than 23 hours—died at from 0.5 to 1.0 day of development. The group of eggs from the "abnormal ring dove" constitute a partial exception. This bird yielded thirteen fertile retained eggs, two infertile eggs delayed 50 and 23 hours, many soft-shelled eggs, all eggs with too little albumen, and (during the period of the production of this series of retained eggs) only six normally laid eggs capable of being tested for development. Only three of these (50 per cent) developed beyond the 2.0 day stage and only one was hatched. In the case of this bird it is evident that some factor, other than delay and also quite irrespective of thick or thin shells, caused the death of nearly all embryos at a very early stage. For purposes of the present paper it is not necessary to identify this indiscriminately acting factor or condition. Even in this group of retained eggs only one egg was capable of more

than 0.8 day of development and this was one of three delayed for the shortest period and one whose shell was thin. The data for the other two groups, however, sufficiently established the fact that the death of the embryos in the retained eggs was associated with the delay.

With the above general considerations of the method and meaning of our tabulation before us it is possible to examine critically the individual cases. Beginning with the "ring doves" it will be noted that in the total of 25 cases the eight cases involving longest delay (60 to 122 hours) all died at 1.0 day or less. Case 9 with 52 hours delay, and with a shell of normal thickness when laid (3.2 mgm. loss per hour) and which remained *soft* until within 24 hours of laying, contained a live embryo at laying. Case 11 is essentially similar; it was retained 48 hours and had a shell abnormally thin (6.5 mgm. loss) even when laid. It too contained a live embryo at laying. Cases 10 and 12 were delayed for periods similar to that of case 11; but their shells were abnormally thick (1.1 and 2.0 mgm. loss) and their germs were dead before the eggs which enclosed them were laid. Case 13 was delayed 34 hours; its shell was of extraordinary thickness, its rate of loss (0.1 mgm.) being only about one twenty-fifth of the normal for eggs of its size. This embryo was dead before laying. Cases 14 and 15 were delayed 29 and 28 hours respectively; both had shells of less than normal thekness, but the one with the thinner shell (8.5 mgm.) had a live embryo, the other (7.5 mgm.) a dead embryo at the time of laying. Cases 16 to 19 were delayed 25 to 26 hours. Case 16 had a thick shell (1.8 mgm.) and a dead embryo; cases 17, 18 and 19 had slightly thickened (2.0 mgm.), normal (4.0 mgm.), or thin (5.1 mgm.) shells and all had live embryos at laying. Possibly, however, case 18 was delayed in the ovary. Cases 20, 21 and 22 were delayed 24, 24 and 23 hours respectively; cases 20 and 21 had normal shells (2.7 and 2.9 mgm.) and were hatched; but case 22 with a much thickened shell (0.9 mgm.) was dead when the egg was laid. Case 23, was delayed probably only 11 hours but it was enclosed in a very thick shell (1.2 mgm.) and was probably dead at laying. Cases 24 and 25, with only 4 to 7 hours' delay and with quite normal shells (3.4 and 3.8 mgm.) were hatched.

In the above group 23 cases were retained for more than 10 hours and 15 of these cases had embryos dead at one day or less. Only 8 of these embryos, or 35 per cent, were alive at the time the egg was laid; these all developed to three or more days and several of them were hatched. From 215 other eggs laid nearest to these retained eggs, by the same parents, 94 per cent of the embryos developed to or beyond the 2-day stage. In this group it is apparent that about 60 per cent of the deaths in retained eggs are ascribable to their retention in the oviduct. It is also clear that the longest periods of delay are associated with the highest percentage of deaths; and that, for comparable periods of retention, the highest death rate falls upon those embryos enclosed in thickest shells.

The 13 cases listed from "an abnormal ring dove" have already been sufficiently discussed. These cases demonstrate that, even in the same individual bird, the retention of eggs in the oviduct may or may not be followed by an increased or continued deposition of shell substance. The two infertile delayed eggs of this bird—not listed on the table—gave rates of loss of 2.5 and 8.8 mgm. per hour. During the past two years (the period covered by our present data) only two birds besides this abnormal ring dove have produced more than one delayed egg capable of developmental tests. Cases 2, 3, 11 are from one of these birds and cases 17 and 25 are from the other.

To the 12 tabulated cases of delayed eggs from common pigeons can be added two infertile eggs; one delayed 36 hours with a normal rate of loss (7.2 mgm. per hour); the other delayed 24 hours with a thickened shell (3.2 mgm.). Cases 39 to 42 of the table were delayed 36 to 120 hours; all had thickened shells and all had dead embryos at laying. Cases 43 and 45 were delayed, certainly in the *oviduct*, for 24 hours; both had somewhat thickened shells. Here it is the embryo within the egg (very small = 12.4 g.) with the (probably) thicker shell (2.2 mgm.) which survived. Cases 44 and 46 to 49 were probably delayed in the ovary, not in the oviduct; if this is a fact, the complete development found in cases 44, 47, 48 and 49 is the expected result; the very early death of case 46 cannot, however, be thus explained. Unfortunately, a measure of the thickness of this shell was not obtained. Case 50 was delayed only 12 hours; it had a normal shell and was hatched. This group of retained eggs indicates—though less definitely—the same relationship of duration of the delay and thickness of the shells to death or survival as has been shown above for the group of ring doves.

From the foregoing analysis of the data it may be concluded that embryos abnormally retained within the oviduct die more frequently when enclosed within thick shells; and also when retained for the longest periods. Further, the amount of development reached at death in the observed cases lies between 0.5 and 1.0-day stages.

The oxygen relations and requirements of the embryo in ante-laying and post-laying development. We may next consider the oxygen relations of embryos encased in thick heavy shells when forced to undergo post-laying developmental stages within the mother's oviduct. The points most directly concerned in this question have apparently never been investigated. It has long been known that the embryo of the fowl, when removed under aseptic precautions from the fresh laid egg, may undergo 2 to 6 days of development when properly held and covered in glass containers (2), (3), (4), (5). Patterson (6) thus succeeded in keeping oviducal stages of the pigeon blastoderm alive—in one case during 33 hours. All of the procedures used by these workers seem to have involved limitations upon the free access of air to the blastoderm or embryo, but the extent of this limitation was not measured in any case.

Measurements made in our laboratory refer chiefly to harmful increased oxygen pressures and to the minimal oxygen requirements of all embryonic stages following laying. The data derived from these studies, however, are capable of a certain amount of projection into the still earlier unmeasured oviducal stages of the embryo. To the above data can be added certain considerations concerning the conditions which apparently should necessarily result from the changes of structure and position involved in egg-building. Finally, to this new information, and to these *a priori* considerations, we can now bring the above described results on oviducal retention as a cause of embryonic death and increased frequency of death when enclosed in abnormally thick shells.

Our own interpretaton of these several facts and considerations is that the shell further restricts an already limited oxygen supply to the germ; that post-laying stages of development utilize ever-increasing amounts of oxygen; that these restrictions of oxygen supply cannot maintain stages with increased oxygen demands in the oviduct but can do so in the air. Such retained embryos therefore probably die of asphyxia. We shall here treat none of these points at length. The facts relating to the oxygen requirements of embryos will be published elsewhere and our present aim is merely to apply these facts to the data already presented above.

In a study carried out quite without relation to the present subject we have determined the minimum oxygen requirements of the several developmental stages in dove embryos after laying. By this we mean the minimum external oxygen pressure capable of sustaining the embryo within a normal or nearly normal shell. We have also studied the effects of various increased oxygen pressures in all post-laying stages enclosed in similar shells. With the exception of embryos aged less than one day all older stages survive increased pressure better than younger stages. Doubtless this fact is related to the circumstance that the later stages have additional and better developed mechanisms of respiratory compensation, such as hemoglobin, circulation, respiratory surfaces (allantois), etc. It is certain, however, that the general trend of the curve formed from the percentage of survivors from ring dove embryos of all ages subjected to high pressures of oxygen (26 to 96 per cent) indicates a probable survival of about 31 per cent for embryos aged 0.0 to 0.9 day. The result actually found for this stage was, however, 46 per cent. This indicates that high pressures of oxygen are less harmful to this stage than the later stages would lead one to expect, and suggests that the still younger oviducal embryo is also relatively resistant to such excess.

A similar curve constructed for survivors of treatment with decreased oxygen pressures (usually 10 to 15 per cent) indicates that the percentage of surviving embryos of this youngest group would be about 85 per cent. But the amount actually found was only 69 per cent. Decreased oxygen pressures are therefore more harmful to embryos aged less than one day than would be predicted from the otherwise uniform trend of the curve for survival at all ages. The still younger oviducal embryos may or may not be similarly sensitive to low pressures of oxygen—the chief difference lying in their smaller absolute oxygen consumption because of their smaller size; but the stage at which the retained embryos actually die (0.5 to 1.0 day) is a stage proved by the preceding studies to be unexpectedly sensitive to low pressures of oxygen. The curves to which we have referred in these paragraphs can be consulted in another publication (7). Similarly constructed curves based on embryos of common pigeons give a wholly similar result and the above conclusions for all post-laying stages are satisfactorily established for both ring doves and common pigeons.

Numerous experiments show that nearly all embryos in fresh laid eggs are able to survive the next following 24 hours of development in 11 to 12 per cent of oxygen. The lowest concentration in which a dove embryo in a fresh-laid egg with normal shell can remain alive during 24 hours is probably between 9 and 10 per cent. The time element is important. In one instance an egg taken 15 minutes after being dropped from the mother survived 8 hours of treatment in nearly complete absence of oxygen (0.15 per cent); and all such embryos readily

survive this treatment during 4 hours. It has also been shown that increased concentrations of oxygen tend to kill selectively those embryos enclosed in the thinner shells. A similar demonstration of the selective survival of embryos in thin-shelled eggs in reduced oxygen pressures can not be demonstrated in our data; but this is probably because some of the embryos in the thin-shelled eggs really die of exposure to the (high) oxygen concentration met with in the air before or after treatment (1). Other details of the data just considered, and not otherwise specifically cited, will appear in a later work on sex.

In view of the several facts cited above it is not to be expected that an embryo could long survive in an egg retained in the oviduct. It follows also that death would more certainly result in an embryo more completely insulated from its oxygen supply by a thick shell. It is probable that some degree of insulation is effected by the membranes and albumen alone. All of our data on the retained embryos suggest asphyxia as the cause of death, and all of the data are rather consistently interpreted on this basis. It is certainly true that the blastoderm's nearest oxygen supply is from the capillaries of the inner oviducal wall, within which the oxygen pressure is probably only such as to supply the cells immediately in contact with the blood and to provide a margin of safety; and, between this supply and the oxygen-consuming embryo there is interposed a layer of oviducal cells, an egg-shell, a shell membrane, and a thick layer of albumen. Of these, the egg-shell has been proved to reduce the oxygen intake from gaseous surroundings and should do the same while in the liquid medium in the oviduct.

It is wholly probable that at the moment each particle of albumen is secreted and laid in place on the yolk it carries with it a quantity of oxygen which serves as a limited reserve to be used by the embryo later while normally still within the oviduct and while completely covered by shell. It is doubtless this same oxygen supply which sustains the embryo in the fresh laid egg during a 4-hour period of oxygen exclusion as cited above.

Discussion. In the preceding section perhaps enough has been said to make it reasonably clear that retention of the egg in the oviduct results in the death of many of the retained embryos; that longer periods of retention and thicker shells are both associated with a greater proportion of deaths, and that the death of the embryo is probably the result of asphyxia. These points will not be further discussed.

The causes of egg retention in the oviduct are only of incidental interest here. In fowls it is known that disturbance of the hen at laying time (separation from nest, etc.) is one such cause and that malformation of the oviduct is another. These same causes are definitely known to be effective also in the case of the pigeon; it can be demonstrated, however, that less than 20 per cent of the retained eggs

obtained by us from ring doves are referable to these sources. It is nearly certain that in these temporary failures of the oviduct some dysfunction of one or more glands of internal secretion is usually involved. These delayed eggs are expelled from the oviduct after the injection of pituitrin, though perhaps less promptly than are premature eggs from a normally functioning oviduct (8). In this connection it may be recalled that our data show conclusively that an egg occasionally remains in the shell-gland during twice, or even three and one-half times, the normal period without receiving more than the normal amount of shell. Cases 7, 8, 9, 11 and 17 are all examples of this. These are also cases from birds which had shown themselves fully capable of producing normal shells within the normal period (about 25 hours) of shell secretion.

We have still to consider the relative proportion of retained eggs to the total number of eggs produced. And also the further question whether the "first" or "second" egg of the pigeon's clutch is more affected by death from these causes. Since the present study is chiefly concerned with the immediate cause and the probable extent of a particular group of embryonic deaths we shall also need to note the percentage of such deaths arising from egg retention. Our data are of course less complete than is desirable, but they seem sufficient for an approximately correct answer to these questions.

Our 54 cases (4 infertile) of retained eggs have been obtained during a period in which about 8,900 other eggs were laid. It would thus seem that about one delayed egg has occurred in each 170 eggs laid—about 0.6 per cent. This calculation, however, requires much revision. First, 15 of the 54 cases are from a single parent, which in this respect is quite unlike any other of many hundreds similarly tested during twelve years; probably the data from this bird should be left out of account. Second, only a part of our collection of birds is examined with sufficient care to make it certain that "first" eggs of the clutch are recognized as delayed eggs. This applies fully to the group of common pigeons; probably the 14 cases obtained from them should therefore be doubled, since in ring doves—for which our data are more complete—"first" eggs are as often delayed as "seconds." Again, 5 of these 14 eggs were probably delayed in the ovary, not in the oviduct. Third, probably not quite all "single" and "first" ring dove eggs have been identified; to the number found probably about 10 should be added. Fourth, a total of 19 eggs known to have been delayed but which were broken when obtained, or were laid with shells too thin to become part of the above tests, must be added to the list. These corrections, and the exclusion of the 15 cases from a single bird, would indicate a total of 72 (or 82) delayed eggs, or one for each 124 (or 109)

eggs laid. Including also the cases from the abnormal ring dove there is one delayed to 102 (or 92) normals. For the ring doves alone, in which there is less doubt as to the actual numbers, a similar or smaller proportion of delayed eggs is found—there being a maximum of 64 (or 49) cases in a total of more than 6,500. One delayed egg per 102 eggs laid is the proportion found here. It is quite possible that the proportion of delayed eggs from the common pigeons is exaggerated by the above method of reckoning and that, in fact, delay there occurs more often in "second" eggs than in "firsts."

The question of a differential occurrence of delay and death for the two eggs of the pigeon's clutch is important in connection with genetic studies and in studies of sex ratios in pigeons. In the group of ring doves 11 of the 25 delayed eggs were "singles;" 3 were "firsts" and 11 were "seconds." The additional 14 eggs of this group which were broken or had envelopes too fragile for test show a similar distribution. It is now necessary to consider the meaning of these "single" eggs. Various observations make it certain that some of these singles are really "first" eggs of a pair—the second of which is suppressed in consequence of the delay of the first in the oviduct. By killing some of the mother birds this was verified in the case of three of the birds which produced "single" delayed eggs. In some of these cases the second ovum is suppressed by resorption in the ovary; in others it ovulates abnormally into the body cavity. This latter situation has been observed once in the case of a delayed egg and repeatedly in other circumstances. Practically all of our observations on ovulation in pigeons confirms the following statement by Patterson (9) for the fowl: "So long as there is an egg in the lower part of the reproductive passage the infundibulum does not clasp the ovum, and a second egg is thus prevented from entering the oviduct." But there are certainly other cases in which a single egg only enters the final growth period; and in these cases it is impossible to classify the eggs otherwise than as "singles." If one-half of the apparent "singles" were really "firsts" there was a total of 8 or 9 "first" eggs delayed in the ring doves, of which at least 5 (possibly all except one) were killed by the delay; and 11 second eggs of which 5 were killed by the delay.

From the "abnormal ring dove" the delayed eggs include 11 singles, 3 firsts and 1 second. The very abnormal and unusual character of the reproduction of this bird does not permit confidence that "single" eggs here are really anything other than singles. This bird was killed for examination of the ovary and showed a single ovum (diameter = 7.8 mm.), not a pair, in the final growth period. In this case "singles" probably represent potential seconds as well as firsts. As has been noted above, the 14 delayed eggs of common pigeons were all "seconds,"

but our observations would necessarily overlook "first" eggs delayed in these birds. It is remarkable that so high a proportion (6 of 14) of delayed eggs obtained for this group were probably delayed in the ovary, not in the oviduct; and that this delay was so frequently of 24 hours' duration.

From the above data, particularly the data from normal ring doves, it appears that the delay of first eggs of the clutch is usually accompanied by the death of the enclosed embryo (from a total of 14 "firsts" and "singles" only 4 survived the delay and only 2 were hatched), and also by the suppression and elimination of the corresponding second egg of the clutch in most cases. From the delay of "first eggs" sex and other genetic ratios are therefore not measurably affected. "Second" eggs, however, are delayed with a frequency apparently equal to that of first eggs, and here no suppression of a subsequent ovulation occurs. This will usually leave unmodified the sex and genetic ratios obtained from common pigeons and other hybrid or mongrel doves; but if such delay occurs in some birds of pure species it would there tend to reduce the proportion of female offspring—in consequence of the predominant production of females from such second eggs of the clutch (10), (11). The present data show, however, that the extent of this effect must be very small. The total proportion of retained eggs is not more than 1:100; and of "second" eggs retained this proportion is only about 1:200; finally, death from abnormal retention in the oviduct occurs in only about one-half of the embryos retained in "second" eggs. If all such second eggs be considered female-producing eggs—though some of them are certainly not—the number of females thus eliminated probably does not exceed 0.3 of 1 per cent. This figure applies of course to a general and relatively large breeding test. For a single individual or a restricted group, in which oviducal retention repeatedly occurs, the sex ratio could be more appreciably affected. As a source of embryonic death, irrespective of sex, the above data and calculations indicate that 0.5 to 0.6 per cent of all dove and pigeon embryos produced are killed at less than one day as a result of retention of the egg in the oviduct.

CONCLUSIONS

Approximately one egg of each 100 produced by a collection of doves and pigeons has been found to be abnormally retained in the oviduct for periods varying between 5 and 122 hours.

The retention or delay of eggs within the bird's oviduct results in the death of about one-half of the embryos in doves' eggs retained during more than 5 hours. About 0.5 to 0.6 per cent of all embryos die as a result of egg-retention in the oviduct. Death usually occurs at or near the 0.5-day stage of development.

Increased frequency of death occurs in the eggs longest delayed and in eggs provided with thickest shells.

Death probably results from an oxygen supply inadequate to the increasing requirements of the post-laying embryonic stages thus retained in utero.

The occasional survival of retained embryos during these early developmental stages is a result of specially favorable respiratory conditions; namely, the presence of a thin shell, or a delay in the secretion of the normal amount of shell.

An egg occasionally remains in the shell gland during two or three times the normal period for shell secretion without obtaining more than the normal amount of shell. More commonly eggs thus retained receive a heavier secretion of shell material.

The retention of a "first" egg of the clutch or pair often or usually results in the suppression of the second egg of the pair. The total result is the death or elimination of approximately one-half of the embryos of both eggs of such pairs from breeding data.

The retention of a "second" egg of the pair suppresses no later ovulation. This retention of a second egg occurs, however, only once in 200 ovulations, and approximately one-half of these retained embryos survive. The selective elimination of so small a number of second eggs can have but slight effect on the sex ratio. Even in pure species where second eggs of the pair are predominantly female-producing eggs the proportion of females to males is affected from this source by probably less than 0.3 of 1 per cent.

BIBLIOGRAPHY

(1) RIDDLE: This Journal, 1921, lvii, 250.
(2) PREYER: Specielle Physiologie des Embryo. Leipsic, 1885.
(3) LOISEL: Compt. rend. Soc. Biol., 1900, lii, 582.
(4) FÉRÉ: Compt. rend. Soc. Biol., 1900, lii, 601.
(5) PATON: Journ. Exper. Zoöl., 1911, xi, 469.
(6) PATTERSON: Journ. Morph., 1909, xx, 65.
(7) RIDDLE: Amer. Journ. Anat., 1923, xxxii, 203.
(8) RIDDLE: Science, N. S., 1921, liv, 664.
(9) PATTERSON: Journ. Morph., 1910, xxi, 101.
(10) WHITMAN: Posthumous works. Vol. ii. Carnegie Inst. of Wash. Pub. 257, 1919.
(11) RIDDLE: Amer. Nat., 1916, l, 385.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XIV. Suprarenal Hypertrophy Coincident with Ovulation

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More than a hundred years ago Meckel (1) stated that the suprarenals of birds and amphibians increase in size during the breeding season. Thirty years later this statement was opposed by Nagel (2) and others, but actual measurements of suprarenals and body weights are given in none of these early discussions of the subject. Stilling (3) made it clear that the suprarenals of rabbits attain their largest size in March and April, but noted that the high suprarenal weight was not then present in all individuals. In more recent years several investigators have reported for various animals an increase of suprarenal tissue—usually cortical tissue—during pregnancy and lactation. In most of these studies also definite measurements are lacking either for the amount of suprarenal hypertrophy or for the total increase in body weight of the pregnant animal. Finally, numerous recent studies have indicated that the suprarenals, especially the cortical tissue, are associated with sex or with the development of secondary sexual characters.

An earlier incomplete study (4) presented good but scarcely sufficient evidence that the suprarenals of common pigeons become enlarged quite coincident with each ovulation period. Sections of these glands suggested that both the medulla and the cortex share in this increase in size. It was further found that the beginning of hypertrophy in these glands was simultaneous with a rapid increase of size in the oviduct and in an ovum in the ovary. Some definite evidence that an increased secretion of the medullary part of the gland occurs in connection with the glandular hypertrophy has since been obtained through the demonstration by Honeywell and Riddle (5), (6) that an increase in blood sugar values occurs in close parallelism with the stages of suprarenal hypertrophy. The present study carries the study of the suprarenal hypertrophy at ovulation to a definite conclusion and extends the earlier investigations to three additional kinds of pigeons. These studies show that suprarenal enlargement in pigeons is definitely

associated, not with season or breeding season in general, but with each particular ovulation period; that suprarenal enlargement is connected not alone with sex but with reproduction; and, together with associated studies (5), (6) it is made probable that the suprarenal medulla is closely involved in these processes of reproduction.

These facts would seem to have a bearing on several important subjects. They point to a close relationship between the suprarenal—probably the medulla—and the ovary, and suggest that this connection is effected through the action of the medullary secretion on the carbohydrate metabolism. Or, since we do not know whether the initial step of increased growth is taken in the ovum or in the suprarenal, it is possible that the ovary elicits a definite response from the suprarenal medulla. Other work by Riddle (7) has shown that ovulation is suppressed in pigeons by injections of insulin, and this suggests that a normal, or an increased, blood sugar value is of real significance in the process of ovulation. In any case the suprarenals are involved in essential processes of reproduction in these animals. Again, Whitman (8) and Riddle (9) have shown that forcing pigeons rapidly through a succession of ovulation periods is accompanied by pronounced changes in egg size and in the sex ratio of offspring of pigeons. On the basis of the present results it is now clear that doves thus forced to lay as many as 30 to 40 pairs of eggs per year would produce the later eggs of such a series under longer and longer periods of suprarenal hypertrophy and presumably of suprarenal hypersecretion. Such nearly continuous hypertrophy and hypersecretion, probably involving both parts of the suprarenal, and certainly affecting almost continuously the sugar level of the blood, demonstrate that these changes in egg size and in sex ratios are at least accompanied by or associated with some alterations in the metabolism of the mother bird. Through one or another of its changes or influences the suprarenals are therefore associated with both sex and reproduction.

MATERIALS AND METHODS. Four kinds of pigeons (*Columbae*) have been used and each of these kinds subdivided into healthy and diseased groups. Diseased groups include all birds bearing Ascaridia, showing tubercles in any organ, having other disease macroscopically recognizable to us, or having enlarged spleens accompanied by livers exceeding a definite size for each kind of pigeon used. The spleen and liver have been found in other work (10) to be the organs most frequently infected with tuberculosis in all kinds of pigeons studied. And this infection was found to be associated with pronounced enlargement of these organs. Marked enlargements of these organs are therefore regarded as probable early stages of tuberculosis; or, at any rate, they offer a sufficient reason for the classification of the birds which

bear them as "diseased" birds. In our earlier communication (4) however, this exclusion of large-livered birds was not done. We have here thus classified all common pigeons with livers exceeding 7.0 grams; all F_1 generic hybrids with livers exceeding 4.2 grams; all complex hybrids and ring doves with livers exceeding 4.0 grams. All other birds are classified as healthy though probably some among them bore disease unrecognizable by us.

Only one of the above kinds of pigeons requires a word of explanation. The "complex hybrids" used were offspring of blond and white ring doves (*Streptopelia*) containing a small admixture (less than $\frac{1}{64}$) of another genus (*Turtur orientalis*).

The birds which supply the data for table 2 and figure 1 were killed at all seasons of the year during a period of 18 months; the period from January to July was covered twice, and rather more than two-thirds of all these birds were killed during this period. The birds represented in table 3 were all killed between June 17 and August 17 of the same year. The birds of the latter series were aged 17 to 183 months. The healthy birds listed in table 2 were of the following ages: Ring doves, 7.6 to 25.9 months; complex hybrids, 8.5 to 25 months; common pigeons; 11.5 to 38.1 months. We can observe no relation of age to suprarenal size in these birds (all mature and all producing eggs, except certain birds of table 3). Whether suprarenal size is here affected by season we are not now able to decide, but our data demonstrate that the differences shown in our tables and curves are not due to the killing of the birds for a particular ovulation stage at a particular season of the year.

The birds were killed by decapitation in nearly all cases and the suprarenals carefully dissected out of the freshly killed bird. Since all of the medullary portion of the gland is not fully enclosed within the cortex in birds a uniform method of trimming the dissected organ for weighing was adopted. Weights as given are therefore very slightly less than the actual weights of the total suprarenal tissue; but the weights as given for all groups, and for all stages, are wholly comparable. The weights of the two suprarenals are combined to give the total weight of suprarenal tissue of each bird.

In one case (healthy common pigeons) a correction for differences in body weight was required on the curves; the uncorrected figures are given in table 2. Reference must be made to body size as related to the data obtained for the various ovulation stages. Obviously the normal weight of a bird is distinctly increased in the "during ovulation" period and for 38 to 42 hours thereafter by the presence in the oviduct of an egg weighing 7 to 20 grams. It can scarcely be supposed that the increase of weight from this source requires a correction of the suprarenal

weight. In the case of the healthy common pigeons, however, the birds utilized in the "during ovulation" stage were undersized despite this accretion to their normal weight. In this group also both non-ovulation stages (108 hours before and 108 hours after ovulation) are based upon undersized birds and a correction of all groups to a common body weight is here probably required.

The chief point in our method was to kill the females at a known or identifiable number of hours before or after ovulation, and then obtain weights of suprarenal and some other organs. The reader will wish to know how it is possible to fix or recognize the exact time interval between the hour the bird is killed and the last preceding or next prospective ovulation. An investigator wishing to repeat these observations will require some data not generally available, including some of our own unpublished observations. In the following paragraphs we undertake to supply the most necessary of such data. The important fact in this connection is that *the hour of ovulation and the hour of egg laying are narrowly fixed in pigeons.* The time required for the passage of the egg from the ovary to the exterior is therefore also a definite number of hours. In common pigeons this time for the second or last egg of the pair is about 42 hours, and for ring doves and the hybrids used here it is about 38 hours.

Common pigeons ovulate the "first" egg of the pair or clutch at about 11:00 p.m.; the "second" at about 8:00 p.m., 45 hours later. Ring doves ovulate the "first" egg at about 12:00 (night) and the "second" at about 7:00 p.m., 43 hours later. For all kinds of pigeons the time between ovulation is figured on the curves as of uniform length, i.e., 44 hours.

From the preceding it is clear that if one knows merely the *day* on which a last egg or pair of eggs was laid one can definitely know the number of hours between the last ovulation and the time of killing the bird for examination. When a bird is killed for such post-ovulation stages care must be taken that a new ovulation stage is not initiated in the meantime, and a pre-ovulation stage thus united with a post-ovulation stage. This is avoided by removing the male mate of the ovulating bird from the cage before the "second" egg is laid, and by removing the eggs or nest in order to prevent the female from proceeding with incubation.

The determination of the time in hours preceding an ovulation is a more complicated matter. The explanations which follow should enable one familiar with this material to test and duplicate our results. When the bird is killed (at a known hour and date) the ovary and oviduct at once supply the data essential for fixing the time of the next ovulation. Ova or yolks of various pigeons begin their final growth

period (lasting close to 108 hours) when of 4 to 5 mm. diameter. The change is indicated by the beginning of deposition of yellow yolk (instead of "white" yolk) in a way wholly similar to that earlier described by us for the fowl (11). During this growth period—which is definitely preparatory for ovulation—the diameter of ring dove follicles (ova) is increased by 2 to 2.5 mm. daily (differing for individuals which produce larger and smaller mature ova or yolks); in common pigeons this daily increase averages slightly less than 3 mm. per day. If, for example, one finds the largest follicle present in the ovary of a common pigeon has a diameter of 11.0 mm., this indicates that the preparation for ovulation has apparently already proceeded during two days—since 6 mm. has been added to the ovum's initial diameter of 5 mm. This would further indicate that it is separated from the hour of ovulation by: 108−48 = 60 hours. But it is known that such a "first" egg will ovulate at 11:00 p.m. on the day of ovulation; and, knowing the hour the bird was killed, it may thus readily appear that the actual time preceding ovulation is 56 or 65 or some other number of hours instead of the roughly calculated 60 hours as given above. For some of the ovulation stages obtained by us it was necessary to kill the birds at night.

Still another check on the determination of the date of ovulation is available from the weight of the oviduct. The weight of the resting (non-ovulation) oviduct (see table 2) lies between 0.5 and 0.8 gram in ring doves, and is approximately 1.0 gram in common pigeons. A ring dove oviduct increases very close to 1.0 gram daily for the whole of the period—108 hours before ovulation to the time of ovulating the "first" egg—but the rate is slightly less for the first half than for the last half of this period. In common pigeons this oviducal increase is probably close to 1.5 grams daily during the first half of the pre-ovulation period and about 2.5 grams daily for the latter half of this period (see curves and table 2). The weight of the oviduct can therefore be used as a basis for determining which of any two days in question is to be the date of ovulation. In a very few instances—and almost entirely in diseased birds—we have found the oviduct enlarged wholly beyond the stage indicated by the ovum or by the known time of the last previous ovulation. But in these cases also we have used the average of the intervals suggested by ovum and oviduct.

In actual practice, the previous record of the normal egg size and yolk size of the particular bird being examined is of further assistance in these classifications, and in our own work these data have been available for our use. It is just possible that in a very few cases we have erred by 24 hours in assigning a bird to its proper place in the pre-ovulation period. The period between the ovulation of the two eggs

of the pair can be exactly measured in all cases; and, although we killed birds in various parts of this period, they are all grouped in the curves as having been obtained im the center of this mid-ovulation period. Individual birds were killed at more stages before and after ovulation than is indicated on table 2. For example, 5 common pigeons were killed at from 3 to 10 hours before ovulation, but these are thrown together in an average of 7 hours before ovulation. Finally, the inspection of the ovary and body cavity may readily and reliably indicate whether an unsuspected ovulation has occurred into the body cavity. In these cases we have found that the oviducal weight is often far from normal, and all such cases have been wholly excluded from these data. All birds which had not ovulated within 30 days and which at the time of killing were not in one or another ovulation stage are also excluded from the curves and the data of table 2. In a few instances birds killed at 108 hours after a known ovulation were found on examination to be also at the point of beginning a new ovulation cycle; these birds are recorded in two places in table 2—with the "108 hour before ovulation" group and with the "108 hour after ovulation" group.

PRESENTATION OF DATA. The data of table 1 make it clear that a study of the relation of suprarenal size to ovulation requires the exclusion or separate treatment of all glands obtained from diseased birds. The presence of tuberculosis or of round worms (Ascaridia) is accompanied by a very great increase of suprarenal size in these three kinds of pigeons. The increase occasionally reaches 500 or 600 per cent in birds dead of disease, though in these cases the glands may have lost all appearance of normal living tissue. Even in birds which have continued to reproduce under these diseases to the time of killing the size increase is sometimes more than 100 per cent. Data are also given in this table for the normal suprarenal size of healthy males belonging to three kinds of pigeons. These figures make possible a comparison with the non-ovulation values (108 hours before or 108 hours after ovulation) of the females of corresponding groups as shown in table 2 and figure 1. A correction for size differences of male and female pigeons is probably fully warranted; after such correction these comparisons indicate that there is but slight sex difference in suprarenal size in pigeons. The male suprarenals seem to be slightly larger in all of the three kinds of pigeons. This is true also for a fourth group of 24 males as compared with the non-ovulating females of that group as shown in table 3. The suprarenals of these males average 15.4 mgm. and their body weight 204 grams.

The principal data on the relation of ovulation to suprarenal enlargement are given in table 2 and in the curves of figure 1. The curves satisfactorily present the various facts in their proper relations. Per-

haps we have attempted to show too much in these curves; but it seems important to emphasize and visualize the fact that the beginning of suprarenal enlargement is coincident with the enlargement of the oviduct and with the beginning of a markedly increased growth of an ovum in the ovary. Growth or enlargement seems to begin simultaneously in suprarenals, oviduct and ovum. We will here designate the 44-hour

TABLE 1

Data on the size of the suprarenal glands of various pigeons in health and disease

BIRD	CLASSIFICATION OF		SUPRARENAL DATA		BODY WEIGHT
	Groups	Conditions at necropsy	Number of birds	Average weight (in mgm. per bird)	
Ring doves	Dead of disease (both ♂ and ♀)	Tuberculous	9	46.0	
		Tuber. + Ascaridia	8	27.8	
		Ascaridia only	7	18.4	
		Other disease	9	16.0	
	Thought healthy killed for necropsy (♂♂'s only)	Tuberculous	13	18.0	
		Tuber. + Ascaridia	2	18.2	
		Ascaridia only	16	17.3	
		Healthy	23	*11.7*	163
Complex hybrids	Thought healthy killed for necropsy (♂♂'s only)	Tuberculous	14	19.2	
		Ascaridia only	18	14.7	
		Other disease	11	15.0	
		Healthy	14	*10.9*	160
Common pigeons	Dead of disease (both ♂ and ♀)	Tuberculous	5	54.8	
		Tuber. + Ascaridia	4	39.2	
		Ascaridia only	9	37.6	
		Other disease	14	41.6	
	Thought healthy killed for necropsy (♂♂'s only)	Tuberculous	8	27.5	
		Tuber. + Ascaridia	1	24.8	
		Ascaridia only	33	26.2	
		Healthy	23	*19.9*	344

interval between the moments of dehiscence of two ova from the ovary the "ovulation period" or mid-ovulation; and this period, together with a preceding period of 108 hours during which the oviduct increases in size and a succeeding period of 108 hours during which the oviduct returns to its former size, will be called the "ovulation cycle."

In each of the three kinds of pigeons shown on the curves the suprarenals of healthy birds killed at points most removed from ovulation

TABLE 2

Summary of data concerning increased weight of the suprarenal glands coincident with ovulation in various pigeons

KIND OF DOVE (NUMBER)	GROUP (NUMBER)	NUMBER OF BIRDS	DATA ON OVULATION PERIOD		DIAMETER LARGEST OVUM (MM.)	AVERAGE WEIGHTS		
			Hours	Period		Oviduct (grams)	Suprarenals (mgm.)	Body (grams)
Ring doves (45)	Healthy (11)	1	88	Before	6.9	1.670	10.8	160
		1	50	Before	10.3	2.418	10.7	153
		2	8	Before	14.7	4.194	12.3	163
		4		During	15.6	5.749	13.0	173
		1	20	After	3.7	4.005	14.2	177
		1	67	After	3.0	1.533	13.4	160
		1	108	After	3.4	0.350	11.6	134
	Diseased (34)	6	108	Before	4.6	0.670	13.1	161
		4	92	Before	6.4	0.963	14.4	153
		2	68	Before	9.1	2.144	13.3	155
		2	31	Before	11.8	2.936	11.1	145
		2	9	Before	13.1	4.265	12.5	155
		10		During	15.6	4.833	14.3	176
		2	3	After	4.3	4.418	13.2	169
		3	63	After	5.1	1.561	12.8	160
		3	108	After	3.9	0.526	12.6	147
Complex hybrids (47)	Healthy (17)	2	93	Before	6.2	1.092	10.6	150
		1	72	Before	8.1	2.020	12.5	161
		1	33	Before	11.7	2.877	14.2	164
		1	8	Before	13.9	3.909	13.2	162
		4		During	14.7	4.760	12.9	177
		3	1	After	3.7	4.845	13.0	159
		3	66	After	5.2	1.437	12.5	152
		2	102	After	5.0	0.856	10.1	157
	Diseased (30)	2	108	Before	5.0	0.756	15.5	145
		3	84	Before	7.1	1.894	15.9	167
		5	38	Before	12.1	2.806	14.5	162
		1	8	Before	15.0	3.430	16.2	157
		6		During	14.9	4.433	13.0	158
		4	62	After	6.0	2.130	11.7	155
		4	85	After	4.2	1.362	14.8	152
		5	108	After	3.5	0.550	13.8	147
Common pigeons	Healthy (27)	4	108	Before	4.9	0.996	16.3	307
		4	43	Before	14.0	4.211	26.3	352
		5	7	Before	18.8	10.951	39.0	364
		5		During	17.5	9.531	35.8	341
		4	16	After	3.7	8.445	24.3	349
		2	56	After	4.5	5.441	23.6	373
		3	108	After	5.0	0.943	16.2	317

TABLE 2—*Concluded*

KIND OF DOVE (NUMBER)	GROUP (NUMBER)	NUMBER OF BIRDS	DATA ON OVULATION PERIOD		DIAMETER LARGEST OVUM (MM.)	AVERAGE WEIGHTS		
			Hours	Period		Oviduct (grams)	Suprarenals (mgm.)	Body (grams)
Common pigeons (82)	Diseased (55)	12	108	Before	4.5	1.311	28.5	322
		5	61	Before	10.5	3.448	28.9	330
		7	27	Before	16.4	6.807	27.1	338
		4	4	Before	18.8	9.283	23.5	344
		10		During	18.1	9.348	27.6	333
		6	19	After	3.8	8.464	24.5	340
		4	49	After	5.2	4.485	34.9	343
		2	67	After	3.5	2.740	19.0	302
		5	108	After	4.5	1.298	21.1	332

are of smaller size than are the suprarenals of healthy birds killed during ovulation or only a few hours before or after ovulation. For the common pigeons—where the data are based on the largest numbers and are probably most accurate—the degree of nearness to ovulation is most closely associated with degree of suprarenal enlargement. In this case too the curve for suprarenal growth and recession is most closely parallel to that of the oviduct. The data of these curves (and of table 2, in which numbers and body weights are given) demonstrate that in these three kinds of pigeons the suprarenals of healthy birds undergo hypertrophy in close coincidence with ovulation. The suprarenals of diseased birds are already enlarged before ovulation and—when thus tabulated in groups—they show no definite enlargement at ovulation. It is possible that a further enlargement occurs also in these (diseased) glands, and that it is merely undetectable by our method of study because of the great variation of suprarenal size in diseased birds. In still a fourth kind of pigeon (table 3) the larger size of the suprarenals in ovulating birds, as contrasted with healthy non-ovulating birds, is shown. In this table all of the six birds found in any ovulation stage are thrown together; and four non-ovulation groups are formed from the 17 healthy birds in non-ovulation stages. In none of these four groups does the suprarenal size closely approximate to the value found for the group killed at or near ovulation.

It is fairly evident from the data of tables 2 and 3 that the differences of suprarenal size as found for the various ovulation stages are not explainable on the basis of differences in the body weights of the various bird groups. Inspection of the data on this point should bear in mind the previously noted fact that the "during ovulation" stage, and for 38 to 42 hours thereafter, the bird is carrying an egg in the oviduct which increases the normal weight of the smaller doves by 7 to 12

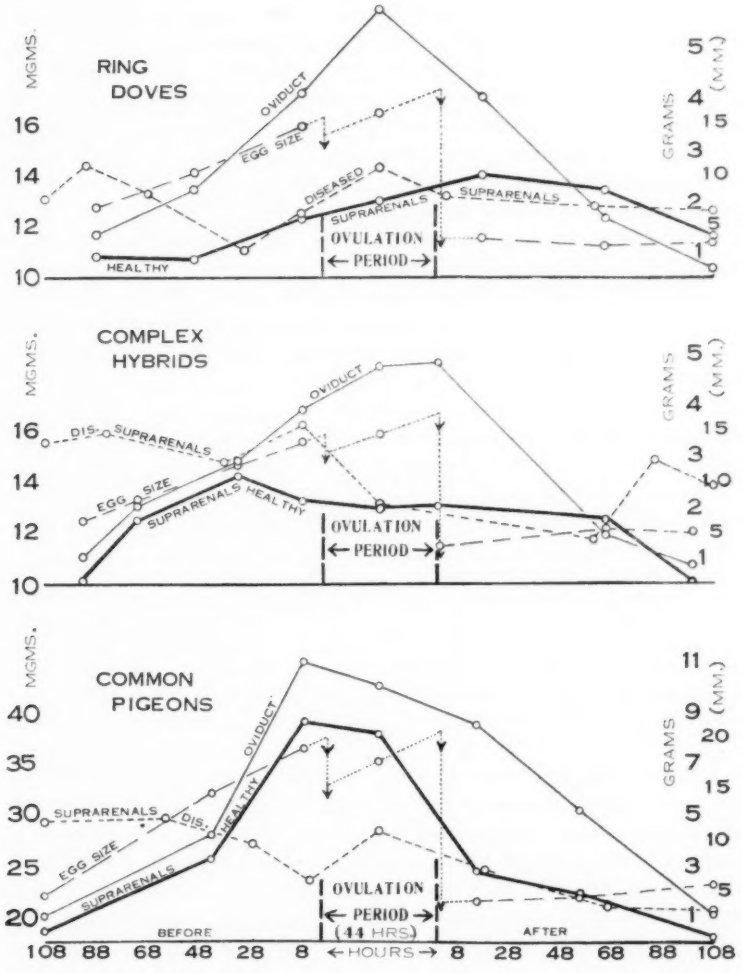

Fig. 1. Curves for size of suprarenals, oviducts and largest ovarian ova of three kinds of pigeons killed during ovulation and at a known number of hours before or after ovulation. The base lines mark off this time in hours with reference to ovulation. The ordinates to extreme left indicate milligrams weight of suprarenals; figures to the right indicate grams weight of oviducts; those to extreme right indicate millimeters diameter of ova.

grams and that of the common pigeons by 14 to 20 grams. It seems that increase of body weight from this source may properly be left out of account in the consideration of the ratio of suprarenals to body weight; i.e., these amounts are deductible from the body weights as given. That there is only a loose correlation of suprarenal size with body size within each group of birds killed at the same ovulation stage is much better seen in our complete data. For example, of the four healthy common pigeons which form the "108 hours before ovulation" stage (table 2) the lowest suprarenal size (11.4) mgm.) was found in the largest bird (324 grams) while the smallest bird (275 grams) had suprarenals of 16.7 mgm. And, within the seven ovulation stages tabulated for these healthy common pigeons, the largest suprarenals are associated with the largest bird of that same ovulation stage in only two cases; the smallest suprarenals are thus associated with the smallest bird in only two cases.

TABLE 3

Suprarenal size of healthy female F_1 generic hybrids (Turtur orientalis × Streptopelia alba) in relation to ovulation

CLASSIFICATION OF GROUPS WITH REFERENCE TO PROXIMITY TO OVULATION	NUMBER OF BIRDS	SUPRARENAL WEIGHT (MGM.)	DIAMETER LARGEST OVUM (MM.)	WEIGHT OF OVIDUCT (GRAMS)	BODY WEIGHT (GRAMS)
Less than 100 hrs. before or after	6	15.0	9.4	2.096	188
30–63 days after; ovary inactive	2	12.2	2.0	0.455	195
14–77 days after; ovary becoming active	5	12.4	5.1	0.356	192
3–12 mo. after; ovary becoming active	4	12.1	4.9	0.194	194
Mos.–yrs. after; ovary inactive	6	13.1	1.9	0.153	190

The percentage increase of suprarenal size at ovulation for the four kinds of healthy pigeons may be calculated and given as follows: If (from table 2) we combine the groups obtained longest before and after ovulation for comparison with the mid-ovulation group plus other birds taken less than 10 hours before or after ovulation, we find the hypertrophy to be 11 per cent in ring doves, 33 per cent in complex hybrids, and 91 per cent in common pigeons. In the fourth group of generic hybrids (table 3) all ovulation stages show an increase of nearly 20 per cent over the combined non-ovulation stages. The mean percentage increase for the four groups is 39 per cent. This mean, weighted for the number of birds compared in the four above groups, is 42 per cent. The actual number of birds used in each of the four comparisons, with non-ovulation figures given first and ovulation stages given last, is as follows: Ring doves, 2 and 6; complex hybrids 4 and 9; generic hybrids, 17 and 6; common pigeons, 5 and 10. Or, a total of 28 in non-ovulation

and 31 at ovulation. Additional comparisons of stages more or less removed from ovulation with the mid-ovulation stage are of course also possible. The curves sufficiently indicate the nature of the result in such comparisons and these need not be calculated here.

The curves showing the changes in weight of the oviduct during the ovulation cycle very clearly indicate its greatest development at or during the act of ovulation. Fairly smooth curves were obtained for the three kinds of pigeons in which this point was studied. It is of course to be understood that the identical birds which supplied the data for suprarenal size also supplied the data for oviducal and egg size.

In presenting the data for increase of egg size (diameter of largest ovarian follicle) we have indicated the measurement actually obtained by points placed on the curves in the usual way. In addition, by means of certain arrows and dotted lines, we have attempted to show more adequately two things which are positively known to occur at the periods indicated. First, that at the point indicated by the arrows at left-hand margin of the "ovulation period" the "first" egg of the pair leaves the ovary and is no longer directly comparable with the egg later found and measured within the "ovulation period." Second, it is the "second" egg only of the pigeon's clutch which is or can be measured at any time within the ovulation period; and this egg continues its growth until it leaves the ovary at the point indicated by arrows at the right-hand margin of the ovulation period. All of the three curves for egg size in the "after ovulation" periods show that the birds killed in these periods contained ova growing either very slowly or not at all. The flatness of these curves throughout this period is conclusive evidence that no birds already beginning a new "ovulation cycle" have been mistakenly placed in these "after ovulation" groups.

Discussion. In the above we have sometimes ventured to use the term suprarenal "hypertrophy" instead of increased suprarenal weight—the thing actually and sufficiently investigated. There is probably no doubt that in these cases we are dealing with a simple functional hypertrophy but we do not wish our use of the term to imply that we have made adequate histological examinations of these tissues. Sections from one or more parts of glands obtained from diseased birds, and from healthy ovulation and non-ovulation stages, have been examined by us; but properly fixed tissues have not yet been obtained nor has an adequate study of the sections been made. Our tentative examination of the sections, however, together with the now obvious fact of the temporary nature of the increase in size of the gland and the plain evidence that this increase is not merely growth, all seem to justify our speaking of this size increase as suprarenal hypertrophy.

Our present interpretation of the sections examined is that both medulla and cortex share in the glandular hypertrophy at ovulation. Acting upon the available evidence that an increased amount of medullary tissue is present, a study of blood sugar values in relation to ovulation was undertaken as a check on this point. Presumably, if an increased secretion of adrenalin occurs at this period this would be accompanied by higher sugar values in the blood. An earlier study of this point by Honeywell and Riddle (5) and a later study by the same investigators (6) have shown that during the "ovulation cycle," and most pronounced at mid-ovulation, there occurs in pigeons a rise and fall in the blood sugar values which is quite parallel to the rise and fall of suprarenal size observed in this study. The evidence obtained from these studies on the blood sugar curve in relation to ovulation therefore affords some evidence that the suprarenal medulla increases the amount of its well-known secretion; and that the amount of this secretory tissue is increased during the few days of proved hypertrophy of the whole gland is thus rendered more probable.

Reference should be made here to those earlier studies which have associated suprarenal hypertrophy with season, breeding season, sexual activity, pregnancy, lactation, or other reproductive period. Also, in the light of the results of the present study we have thought it advisable to examine the evidence cited by several investigators for suprarenal hypertrophy in other animals in one or another stage of reproduction—particularly in pregnancy and in lactation. A very few points resulting from this examination should also be mentioned here.

At the outset of a consideration of the point last mentioned above we meet the fact that in these several investigations the special relation of ovulation to suprarenal size has been overlooked; and, if the relation found by us in four groups of pigeons also holds in mammals, this circumstance alone would probably prevent an entirely correct measure of the extent and of the frequency of hypertrophy. For presumably some ovulation stages and some non-ovulation stages would have been taken as normals for comparison with one or another stage in pregnancy or in lactation; and either or both such stages might have been taken as normals in histological studies of suprarenal changes during pregnancy. It is evident that investigations on mammalian suprarenals should now determine whether and what suprarenal changes at ovulation also occur in mammals; and if these do occur only mammalian suprarenals in non-ovulation stages should be compared with glands in pregnancy, ovulation, lactation, or other stages of reproduction. A second general point of equal or greater importance is the probability that in at least some of the earlier investigations a number of diseased or infected animals were included in the study. Finally, and of a different significance, is

the consideration that variations from these two sources in the material examined by different investigators may explain the opposed results sometimes reported for the same animal.

Meckel (1) was probably wholly correct in his observation that the suprarenals of birds and amphibia enlarge during the breeding season; the animals are then near to ovulation periods. Unfortunately Meckel did not leave the measurements necessary to convince others. Nagel (2) particularly has opposed Meckel's views stating that, among the birds, he "examined the pigeon, guinea hen, fowl and duck during and after the breeding season and always found the adrenals of similar color, size and consistency.' Among mammals this observer also "studied the adrenals of pregnant sows, rabbits and cats and these were not different from organs taken from non-pregnant animals or from animals in the breeding season." Gottschau (12), however, later noted that the suprarenals of rabbits do undergo changes of volume during pregnancy. And Stilling (3) reporting upon 150 rabbits noted a seasonal varition with highest suprarenal weight (body weights and gland weights not given; histological study) in March and April. Since Nagel's publication the hypertrophy of the suprarenals has been reported in several mammals by various workers; and the present work demonstrates the hypertrophy which he was unable to find in pigeons. It seems probable that in 1836 Nagel was unable to make a very complete study of all the several animal species listed by him.

Stilling conservatively writes that he has not cleared up all the difficulties connected with suprarenal enlargement in rabbits on the basis of season, noting that heavy glands were not found even in March and April in all individuals killed at that time. In this same study Stilling discovered the remarkable "summer" cells of *Rana esculenta*, and made it clear that in this animal certain marked and definite histological changes occur in the suprarenals at about the time of "the pairing of the frogs." It was further noted that the changes in ovary and suprarenal were essentially simultaneous and that the hypertrophy begins in the autumn months. Glynn (13) believes that similar seasonal changes occur in human beings; but definite data on this matter are wholly lacking. Porier and Charpy (14) report some facts concerning the influence of sexual activity on the suprarenals, and Walter (15) concludes from examination of cadavers that fluctuations in ovarian size are accompanied by similar changes of suprarenal size.

In addition to the cases already mentioned suprarenal hypertrophy during pregnancy has been observed by Guieysse (16) and Verdozzi (17) in guinea pigs; these authors and Kolmer (18) find hypertrophy or proliferative changes in the cortex; body weights and gland weights

are either omitted or rather insufficiently given in these studies. Verdozzi notes, however, that at the end of pregnancy suprarenal size was only slightly above normal and that the enlargement was not constantly shown during pregnancy. He further suggests that this hypertrophy "probably changes at fixed epochs" during pregnancy. In explanation of these epochs it seems to us that the possibility of ovulation during pregnancy in the guinea pig should not be overlooked. Herring (19) recently reported that the suprarenals of albino rats undergo slight hypertrophy during pregnancy. Body weights and gland weights are given, but the uterus and its contents were not included in the body weight of the pregnant rats. Since the increase in suprarenal weight was only 12 per cent, and the extra large glands of *two* of the nine pregnant rats examined contributed much or most of the total increase, it is clear that the increase found is slight and possibly represents merely growth in adjustment to the increased size of the animal. Stoerk and v. Haberer (20) have, however, adequately shown that suprarenal hypertrophy occurs during pregnancy in a number of species; and that although the chief hypertrophy is found in the cortex it occurs also in the medulla.

That a similar hypertrophy occurs during lactation in guinea pigs has been indicated by Verdozzi (17) who reports the hypertrophy confined to the cortex. Kolmer (18) very briefly refers to certain periods of cell proliferation in the pregnant and lactating guinea pig and seemingly raises a question as to whether these cyclical changes are really related to pregnancy or to periods of rut. Our present results suggest that further exact knowledge of the ovarian cycles in guinea pigs during pregnancy and lactation will assist in the interpretation of the results obtained by Verdozzi and of Kolmer.

From the above consideration of our own work in relation to that of others it can be concluded that suprarenal hypertrophy probably occurs in more than one phase or stage of reproduction; that the hypertrophy at ovulation is probably of widespread occurrence in vertebrates; and that the hypertrophy during both ovulation and pregnancy probably includes cortical and medullary portions of the gland. We consider it highly probable that once it is specially looked for the hypertrophy at ovulation will be more easily demonstrated in many animals and found in a more pronounced degree than is the hypertrophy accompanying pregnancy.

Two points remain to be considered. The first is concerned with sex differences of normal suprarenal weight. In the present study probably sufficient data are presented to show that male pigeons have slightly larger glands than have the non-ovulating females. Certainly the male glands are as large as those of the female. In the rat, however,

the studies of Hatai (21) and of Jackson (22), (23) make it certain that the female suprarenals are larger, and that this difference in size begins to appear at about the age of 40 days. Whether the direction of this sex difference of suprarenal size bears any relation to the degree of suprarenal hypertrophy attainable during ovulation or pregnancy is a question now unanswerable. But we have been able to demonstrate very evident hypertrophy at ovulation in the female pigeon—which bears a smaller suprarenal than the male; while Herring found only slight hypertrophy in pregnant rats, and the slight amount of increase found there is possibly accounted for by failure to include the weight of the gravid uterus in the calculation.

The last point for consideration here is the bearing of the present results upon our own earlier studies on sex and reproduction in pigeons. The present work was undertaken primarily to obtain an explanation of some of the phenomena observed in studies on sex and sex ratios. Whitman (8) and Riddle (9, and earlier papers) have observed that pigeons made to ovulate eggs in rapid succession (crowded reproduction, or reproductive overwork) soon begin to produce predominant numbers of females. Riddle found also that the ova (yolks) produced under this treatment became larger. During the nearly twelve years since this latter result was reported several lines of evidence have been obtained all of which indicate that the procedures involved in "reproductive overwork" effect a lowering of the metabolism within the ova thus produced; that femaleness generally is characterized by a lower level of metabolism than is maleness; and that the lower metabolism forced upon the maturing ova in the initial stage of development is able to force certain ova which would otherwise produce males to develop into females instead. In some of these latter cases it is probable that the processes of maturation in the egg have been thus controlled; but of much greater interest and importance is the accumulated evidence that many eggs of an original sex inclination and constitution have been forced by the conditions accompanying "crowded reproduction" to develop into the opposite sex.

The demonstration of a pronounced suprarenal hypertrophy at each recurring ovulation period is of real importance to some aspects of the above conclusions. Females made to pass through 30 to 40 ovulation periods, and yielding twice that number of eggs per year, are now known to produce these eggs under practically continuous suprarenal hypertrophy which has already been demonstrated to affect at least the carbohydrate metabolism of the mother bird as shown by higher than normal blood sugar values at each recurring ovulation. That the suprarenal hypertrophy becomes practically continuous for such birds is readily calculated. It begins at 108 hours before ovulation, includes

the 44-hour ovulation period, and (when a new ovulation period is not initiated at this point) comes to an end only after another 108 hour period; these 260 hours are but slightly less than 11 days. There are only 34 such periods in a year.

Both parts of the suprarenal are probably involved in the suprarenal hypertrophy The present and associated studies, and some investigations by others indicate that the suprarenal medulla is closely connected with reproduction and with some phases of metabolism. Numerous investigations of others have shown that the cortex is intimately associated with sex—with the gonad and with the development of secondary sex characters. If both parts of this gland are persistently held at abnormally high functional levels the ovary and ova, which stand in so close a functional relation to the suprarenal, can scarcely be thought to have their metabolism unaffected. Just what the nature of the response of the ovum to this change is or may be is a question that can not now be answered, particularly since we here deal with a germ cell on the one hand and with somatic organization on the other; but the important fact is that a pronounced and almost continuously acting somatic change is introduced and to this change one is assured that even a germ cell must make one or another response.

CONCLUSIONS

In four kinds of healthy doves and pigeons the weight of the suprarenal glands has been found to increase at each ovulation cycle and the point of greatest increase is coincident with the act of ovulation. The mean size increase found in the four groups was approximately 40 per cent.

The diseases commonly met with in our birds usually produce very marked suprarenal enlargement; and all birds with disease recognizable to us have been separately classified and tabulated as diseased birds. Without this separation of healthy and diseased birds a definite relation of suprarenal hypertrophy to ovulation could scarcely be obtained from any reasonable number of birds by our method of study since the glands of diseased birds are extraordinarily variable in size.

Previous studies on suprarenal hypertrophy during pregnancy and lactation have overlooked hypertrophy at ovulation; if the latter occurs also in mammals this is a possible source of error in many results reported. The changes in the suprarenals of mammals at ovulation should be investigated.

The suprarenals of male pigeons are probably slightly larger than those of females even after fully correcting for differences in body weight.

When doves are provided with heated quarters and are made to reproduce throughout the year, as were the birds used in this study, it does not appear from the considerable data at hand that season is in itself associated with any change in suprarenal size. If such seasonal changes do occur they do not affect the results reported.

Adequate histological studies have not yet been made but it is probable that both medulla and cortex share in hypertrophy at ovulation.

These results make an important contribution to our definite knowledge of the conditions associated with previously observed changes in size of ova, of sex ratios, and of sexual development in the offspring of pigeons made to reproduce rapidly and continuously throughout the year. They demonstrate that some element of a new and intimate environment is provided for ova thus made to develop under prolonged and nearly continuous suprarenal hypertrophy.

BIBLIOGRAPHY

(1) Meckel: Abhandl. a. d. Menschl. u. Vergl. Anat. u. Physiol., Halle, 1896.
(2) Nagel: Müller's Arch. f. Anat. u. Physiol., 1836, 365.
(3) Stilling: Arch. f. Mikr. Anat., 1898, lii, 176.
(4) Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 280.
(5) Honeywell and Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 377.
(6) Riddle and Honeywell: This Journal, 1923, lxvi, 340.
(7) Riddle: Proc. Soc. Exper. Biol. and Med., 1923, xx, 244.
(8) Whitman: Carnegie Inst. of Wash., Publ. 257, 1919, vol. ii.
(9) Riddle: Amer. Nat. 1916, l, 385.
(10) Riddle: Journ. Infect. Dis., 1921, xxix, 544.
(11) Riddle: This Journal, 1916, xli, 387.
(12) Gottschau: Sitz. Ber. d. phys. med. Ges., Würzburg, 1882, xvii, xviii.
(13) Glynn: Quart. Journ. Med., 1911–12, v, 157.
(14) Porier and Charpy: Traite de Anat., v, 2 fasc.
(15) Walter: Frankfurter Zeitschr. f. Path., 1922, xxvii, 276.
(16) Guieysse: Journ. d. l'Anat. et de la Physiol., 1901, xxxvii, 435.
(17) Verdozzi: Policlin., Roma, 1915, xxii, 481.
(18) Kolmer: Zentralbl. für Physiol., 1912, xxv, 1009.
(19) Herring: Brit. Med. Journ., 1920, ii, 886.
(20) Stoerk and von Haberer: Arch. f. mikr. Anat., 1904, lxxii, 481.
(21) Hatai: Amer. Journ. Anat., 1913, xv, 87.
(22) Jackson: Amer. Journ. Anat., 1919, xxv, 221.
(23) Jackson: Amer. Journ. Anat., 1913, xv, 1.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XV. Increased Blood Sugar Coincident with Ovulation in Various Kinds of Pigeons

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In earlier studies by one of us (1), (2) it has been shown that the suprarenal glands of the pigeon undergo a marked hypertrophy coincident with ovulation. This hypertrophy begins about 108 hours before the next prospective ovulation, and is also quite coincident with the establishment of a new and much increased rate of growth in the ovum next to be ovulated; and is further coincident with the beginning of a remarkable growth or hypertrophy of the oviduct. Since enlargement in these three distinct organs apparently begins at the same time, it has thus far proved impossible to obtain evidence as to which, if either, of these is primarily responsible for this series of interrelated events.

The fact of suprarenal hypertrophy raises the question whether the hypertrophy is shared by both the medulla and cortex and whether each or only one of these parts actually acquires increased functional power. Since adrenalin is secreted by the medulla, and an increase of adrenalin in the blood results in an increase in the amount of sugar in the blood, it is possible to learn from a study of the blood sugar curve whether an increased action of the medulla probably occurs in connection with the enlargement of the gland. Adrenalin is of course only one of the factors which determine the general level of glucose in the blood; but perhaps the "ovulation period" may provisionally be regarded as an "emergency" in Cannon's sense, and thus a period during which an increased output of adrenalin sustains a higher level of sugar in the blood. Whatever may be the relation of the adrenalin output to the amount of blood sugar present during these recurring periods, it was thought of importance to make measurements of the blood sugar during the ovulation cycle which is a cycle accompanied by suprarenal enlargement. It is the result of this particular study which is reported here.

Any metabolic change, of any nature, which is repeated with each ovulation cycle in the pigeon is of much importance to central problems of sex in addition to its bearing on reproduction. For it has earlier been shown by Whitman (3) and Riddle (4) that rapid and continuous ovulation in the pigeon results in a profound modification of the sex ratio (and of sex itself, according to Riddle), and ultimately on longevity and viability of the offspring. Other studies by Riddle have identified these sex and developmental changes or transformations with metabolic changes in the ova and in the female parent producing the ova. As a result of the present study it becomes clear that a pigeon forced to continuous egg-laying is a bird in which the sugar metabolism of the parent bird is almost continuously disturbed and held at an abnormal level. Whether this disturbance signifies an increase or a decrease in the rate of the burning of carbohydrate is a question essentially untouched by this study; but the fact that a change or modification in the carbohydrate metabolism occurs is established.

An account has elsewhere been given (5) of one of three series of determinations made in order to learn whether the blood sugar increases at ovulation in a way at all smilar or parallel with the increased weight of the suprarenal gland. In the present paper all of the data bearing on this subject as obtained in three series of determinations will be presented. The only previous work in which an association of high blood sugar with any aspect of egg production has been suggested was that of Scott and Kleitman (6) in studies on the frog's blood. These authors concluded that during the months of February, March and April the "sugar content of the blood of males is lower than that of females, and it is lower in the females with poorly developed eggs than in those with well filled ovaries." These authors further state that their results "indicate that this condition (value of the blood sugar) is directly related to the condition of the eggs." It will be seen that the present study definitely relates the increased sugar values to their proximity to ovulation.

METHODS. *The care and handling of the birds.* All birds were kept with their male mates in well-lighted pens of adequate size and under such other conditions as have been demonstrated to permit practically continuous egg-laying throughout the year, and from year to year. Accurate egg-laying records are kept for all of the birds of the whole collection from which the birds used in this study were chosen. The birds used were not permitted to incubate after laying, but the males were sometimes removed at the time of one ovulation period in order to prevent the almost immediate resumption of a new cycle of growth in ova, oviduct and suprarenals

The common pigeons and the hybrids used were not particularly tame birds. Before any blood samples were drawn from them these birds were therefore caught and handled by the operator, and kept close at hand during a preliminary period in order to minimize danger of a rise in blood sugar due to fright when samples of blood sugar should be drawn. The ring doves were quite tame, were accustomed to handling, and did not seem to require the above precautions. All birds were caught and brought into the laboratory where they were kept in very small cages for at least two hours before samples were drawn. Two samples from the same bird were never drawn at less than a two-hour interval, nor in less than two hours after the last previous handling of the bird for any purpose. The "scragglies" used are a mutational form of common pigeon which shows conspicuous epidermal anomalies, and are separately classified here because they have consistently shown lower values of blood sugar than do ordinary common pigeons.

Special methods and technique. Maclean's micro-method was used and duplicate determinations were usually made on each ovulation stage studied. For the few cases in which these determinations did not check to within 10 mgm., additional determinations were made in most cases. In order to obtain twice or three times an exactly equivalent ovulation stage the determinations made upon a single bird (series I) were sometimes carried out over a period of three or four weeks. Details concerning the time interval (44 hours) between the ovulation (mid-ovulation) of the first and second eggs of the pair produced by doves and pigeons, and also the data concerning the period of beginning suprarenal hypertrophy and of the growth of the ovum, may be found in the preceding paper of this series (2).

The samples were drawn by needle-puncture of the heart. This occasionally resulted in the death of the bird. While the samples were being drawn the bird was encased in a close-fitting cloth jacket made for this purpose. Other details of the method followed here are those earlier used and described by Honeywell (7). In series I we obtained data for three or four different periods in the ovulation cycle upon the same individual bird; the several birds of the group therefore supply data for a still larger number of periods in the cycle (see curve). This method of obtaining these particular samples required of course that the bird be not killed after drawing the earlier samples and here it was impossible to verify an expected pre-ovulation stage at autopsy. The autopsy was usually unnecessary for a definite fixation of the mid-ovulation stage and for the three or four days preceding this stage if a succeeding ovulation occurred in less than four days thereafter. Near the close of this first series of determinations it was learned, however, that the needle-puncture of the heart occasionally *suppresses* the

ovulation of ova which are very nearly ready to ovulate and causes their regression in the ovary. In other rare cases the heart-puncture, or the heart-puncture and associated handling and treatment, resulted in the passage of the ovum into the body cavity instead of into the oviduct. In the light of these facts, and of our later results in which we were able to exclude all uncertainty, it is wholly probable that data for one period, as obtained from one of the common pigeons and from one of the "scragglies" used in series I, were not properly classified.

PRESENTATION OF DATA. The main results of the investigation are sufficiently shown in a condensed form in table 2 and on the curves of figure 1. A more detailed presentation of the results from series I has been given elsewhere (5) and the details of series II (hybrids) are adequately shown by the curve. Table 1 fully presents the results from series III, which was solely concerned with the difference of sugar values measured at mid-ovulation (i.e., after the ovulation of the "first," and before the ovulation of the "second" egg) and at another point removed from ovulation at which the bird was killed so as to fix the ovulation stage beyond doubt. The results obtained from this series are highly consistent and leave no doubt that in these two groups of birds blood sugar was distinctly greater at the ovulation period than at points separated by more than 48 hours from it.

The summary given in table 2 shows that in all of the four kinds of birds, and in all of the six groups of birds studied, there is a greater amount of sugar in the blood at the period of ovulation—i.e., within the interval which elapses between the separation of the two ova from the ovary—than at points more distant than 36 hours from the beginning or end of that period. The individual exceptions within the groups are very few in number, and are probably the result of uncertain identifications of the periods, particularly in series I. Moreover, the sugar values obtained for the six or seven additional groups of birds for whom determinations were made only at mid-ovulation, or only at a point removed from the ovulation period, are in all cases (considered as groups) of a magnitude corresponding to that of their respective periods. The averages of the four kinds of birds studied in all of the three series are all in the same sense. The mid-ovulation stage and stages more than 36 hours from ovulation respectively, are: For common pigeons, 208 and 183 mgm. per 100 cc. of blood; for scragglies, 186 and 160; for ring doves, 176 and 149; for hybrids, 213 and 171. The mean values obtained for all birds in all groups are 196 and 166. The mean values obtained for 32 individual birds whose sugar was determined in both divisions of the ovulation cycle are 194 and 167. It is clear that all groups of birds show considerably increased blood sugar values at the period of ovulation. Mid-ovulation values compared with values ob-

TABLE 1

Details of the blood sugar determinations of series III

	BIRD NUMBER	STAGE IN RELATION TO OVULATION AND AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)		
		Before or after ovulation (hours)		Mid-ovulation period
		108-49	48-0	
Common pigeons	1	185		(205)*
	2	183		203
	3	178		193
	4	178		198
	5	173		198
	Average	179		199
	6	175		
	7	183		
	8	183		
	9	195		
	10	(195)		
	Average	186		
Ring doves	1	153		198
	2	115	123	(140)
	3	170		180
	4	155		(160)
	5	153		(150)
	6	143		170
	7	163		188
	8	163		180
	9	160		193
	10	148		193
	11	163		188
	Average	153	123	176
	12	155		
	13			190
	14			(155)
	15			(195)
	16			(185)
	Average	155		181

* Parentheses enclose single unchecked determinations.

tained from 108 hours before and 108 hours after ovulation indicate that the blood sugar at ovulation is increased by rather more than 20 per cent.

TABLE 2

Amounts of blood sugar obtained within, and apart from, ovulation periods in various kinds of birds

SERIES	KIND OF PIGEON	NUMBER OF BIRDS	PERIOD AS RELATED TO OVULATION AND AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD	
			Mid-ovulation	36 + hours before or after ovulation
I	Common pigeons	4	216	185
		1	220	
	Scragglies	4	186	160
		1		120*
	Ring doves	6	172	140
		1	185	
		2		158*
II	Alba-orientalis hybrids	2	213	185
		3		161
III	Common pigeons	5	199	179
		5		186
	Ring doves	11	176	153
		4	181	
		1		155
Average	Common pigeons	10	208	(14) 183
	Ring doves	22	176	(18) 149
	Scragglies	4	186	(4) 160
	Generic hybrids	2	213	(5) 171
Means, all birds in all groups		38	196	(41) 166
Means, identical birds, both stages		32	194	(32) 167

* Neither mid-ovulation nor more than 36 hours removed from it; classed with neither group in averages.

The curves supply the evidence that there is a gradual rise of the blood sugar value beginning at or near 108 hours before the ovulation period, reaching its maximum in this period, and again undergoing gradual decline. In all these respects the blood sugar curves resemble

the curves for suprarenal hypertrophy earlier described by Riddle (1), (2).

Only in series I and II were data obtained at intervals permitting their arrangement in the form of curves. A curve for the "scragglies" of series I is omitted. It is a less regular curve than those presented, but the mid-ovulation value is higher than any other point in the curve. The data for the "scragglies" are defective. Two of these birds were killed by the first heart-puncture and only unchecked values were obtained. In addition, one bird of this group probably failed to ovulate properly because of heart-puncture in a way similar to that of one common pigeon whose effect on the uppermost curve of figure 1 may next be noted.

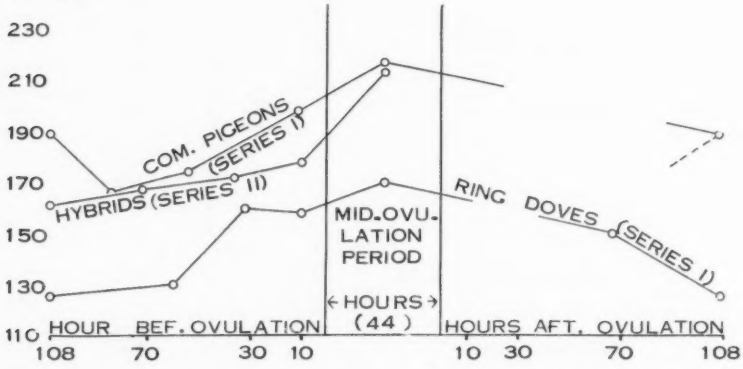

Fig. 1. Curves showing average blood sugar values obtained from three kinds of pigeons during ovulation and at a known number of hours before or after ovulation. The base line marks off time in hours. The ordinates represent milligrams sugar in 100 cc. of blood. For explanation of the ends of the curve for common pigeons see text.

Only three of the five common pigeons used in series I are represented in the extreme ends of the curve (108 hours before and 108 hours after ovulation), and one of these had a very high blood sugar (228 mgm.) which is probably wrongly classified because, as noted above, the correct stage could not at the time be verified by autopsy. This bird was five days removed from the last preceding ovulation when two samples were drawn; after receiving these two heart-punctures she did not again ovulate until another five days had passed. We are thus forced to record these values at both points farthest removed from ovulation though they probably belong to a stage very near to ovulation—an ovulation suppressed by the heart-punctures. The extreme ends of the curve for this group are probably high and irregular for this reason.

The curve for the hybrids (*Streptopelia alba* × *Turtur orientalis*) is given added significance by the circumstance that 31 non-reproducing females, sisters of birds used for curve (but with no ovulation during the previous 60 to 350 days, and therefore not used in the present study), were found to give an average value of 161 mgm. sugar; and from the further fact that the highest value obtained from any of these 31 individuals was 205 mgm., though both of their sisters examined in the mid-ovulation stage showed still higher values.

The curve for ring doves includes the data obtained from the five individuals which at autopsy proved to be free of discoverable disease (tuberculosis, Ascaridia). The curve is less smooth when the diseased birds are included but is otherwise quite similar.

The data for series I were obtained in March; for series II in June to August; for series III in September. A fourth series was attempted in late September and in October but unexpected effects of the onset of cold weather, while our birds were unprotected from the cold, became apparent and it was learned that soon after the beginning of cold weather both the ovulation and non-ovulation blood sugar values are definitely decreased. This effect of cold was therefore further studied, and all results obtained at that period, including partial data for a fourth series of determinations on the present subject, are being prepared for publication.

All of the birds used in this study were killed after, but not in all cases immediately after, the last blood samples were drawn, and the weights of their suprarenals obtained. The notable disturbing effects of disease on suprarenal size, and our failure to find time to kill several of the birds immediately after taking the last blood sample, serve to complicate the data obtained in our attempt to examine this question. Similar attempts to learn whether the thyroids of the bird undergo size-fluctuations coincident with this sustained rise in the blood sugar curve, and whether a relation exists between thyroid size and the non-ovulation blood sugar values of different birds of the same kind, have also proved too complicated for present action here.

SUMMARY

Each ovulation period in the pigeon is accompanied by an increase of approximately 20 per cent in the amount of sugar in the blood.

The sugar content of the blood begins to increase at about 108 hours preceding the ovulation of the first egg; it is then maintained at the highest level until the second ovulation 44 hours later; following this period it probably gradually declines to the normal non-ovulation level in an additional 108 hours—unless new ova meanwhile begin their final period of rapid growth and a new ovulation cycle is begun.

The curve which thus describes blood sugar values as related to ovulation is essentially similar to the curve for suprarenal hypertrophy at ovulation earlier described by Riddle.

The above results apply to various species of doves, pigeons and their hybrids.

Female pigeons forced to rapid and continuous ovulation throughout the year are thus forced to an almost continuous maintenance of their bodily functions—and to the production of their eggs—under altered and unusual conditions as regards their carbohydrate metabolism.

BIBLIOGRAPHY

(1) Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 280.
(2) Riddle: This Journal, 1923, lxvi, 322.
(3) Whitman: Carnegie Inst. of Wash. Publ., 257, 1919, vol. ii.
(4) Riddle: Amer. Nat., 1916, l, 385 (and earlier and later papers).
(5) Honeywell and Riddle: Proc. Soc. Exper. Biol. and Med., 1922, xix, 377
(6) Scott and Kleitman: This Journal, 1921, lv, 355.
(7) Honeywell: This Journal, 1921, lviii, 152.

THE MINERAL METABOLISM OF THE LACTATING AND DRY GOAT[1]

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In the work of Doctor Forbes and his co-workers (1) and the earlier work of Hart and his associates (2) it has been demonstrated that milking cows receiving a ration of grain and dry hay, with and without mineral supplements, are generally brought into a decided negative calcium balance.

Meigs and his associates (3) have noticed a similar response, but are inclined to interpret the negative balance of calcium as only temporary and due to a nervous disturbance induced by the conditions under which the animal had to live during the course of the experiment.

Hart and his associates (4) have drawn conclusions from their work with goats that green feed affects a better utilization of calcium than dry feed. They state that "apparently there is something having its source in fresh green materials, which controls or assists calcium assimilation." Hart (5) has also reported evidence secured with milk cows that it is possible to maintain calcium and phosphorus equilibrium and even a positive balance of these elements on a ration of grain and dry alfalfa hay—the hay having been cured under caps. These results are contrary to those of Forbes and his co-workers (1) and even their own later findings (6) when they obtained a negative calcium balance with milking cows on a similar ration. The alfalfa hay in this case was cured in windrows for four days. One observes from their data that the cows that gave a positive calcium balance were in a rather late period of lactation, while those that produced a negative calcium balance were in a much earlier period of lactation, but they conclude that "these differences in effect of the two alfalfa hays may be attributed to a difference in the degree of destruction during the curing process of the vitamin assisting calcium assimilation."

The factors influencing calcium assimilation are not only of scientific importance, but they are important from a practical standpoint in that they may lead us to better and more economical farm practices.

[1] Published with the permission of the Director of the Ohio Agricultural Experiment Station, Wooster, Ohio.

As the goat is increasingly becoming one of the sources of the milk supply in the industrial centers among our foreign population, and as the value of the milk, especially in infant feeding, is becoming better known, it is very important then that we should know something of the ability of the goats to assimilate food and especially minerals.

Plan of the experiment. In this, our first work with goats, we have included the complete mineral balances with lactating and dry goats. The lactating goats received in successive periods respectively, dry millet hay, dry millet hay plus precipitated bone flour, and green millet (hay) plus precipitated bone flour, in addition to a grain mixture in each case; while the dry goats received green millet (hay), green millet (hay) plus precipitated bone flour, and dry millet hay plus precipitated bone flour, in addition to the grain mixture in each case. Our object in the experiment was to find, if possible, what effect a mineral supplement, when added to a grain and dry grain hay ration, would have upon the balance of minerals, and what effect the change from dry to green hay and vice versa would have upon the mineral balances. We also wanted to know whether the mineral outgo of the goat was similar to that of the cow.

Experimental. We used for this experiment four mature goats, two lactating and two dry. They were of mixed breed and the lactating goats were fair milkers. No. 1 was fresh April 1, 1921, while no. 2 was fresh June 1, 1921. No. 3 was a very poor feeder, the effects of which are shown in the results and hence was removed from the experiment at the close of period I. No. 1 declined in milk yield so rapidly that she was removed from the experiment as a lactating animal and throughout periods II and III she was a dry goat. No. 5 had just freshened (July 15, 1921) five days before the preliminary trial for period II. The goats were not bred. The experiment was begun July 8, 1921.

The goats were confined in metabolism crates which were originally designed for hogs. No alterations of these crates were found necessary, except raising one side of the feed box.

The millet hay used in the experiment was grown on the Station farm. The seed was sown in plots at intervals of one week for ten weeks in order that the hay fed might be of the same maturity for all periods. Weather conditions affected this scheme to some extent. The hay was cut in the afternoon of each day, one part was fed green, while the other part was dried in the sun from two to three days. In case of rain it was dried in a warm air chamber at about 55°C. Samples for the moisture and an amount necessary to make up the composite sample for the period were taken each day. The hay (green and dry) was weighed out in the afternoon, as near feeding time as possible, for the afternoon and morning feedings, and the morning's feeds were then placed in the refrigerator (about 2°C.) until feeding time next morning.

The amount of feed fed was determined in advance during a preliminary trial. An attempt was made to feed the green millet in period III on the same dry matter basis as the dry millet in period II but they would or could not consume that amount and as a result the green hay was fed practically *ad libitum*. The goat is rather stubborn, and sometimes an animal would refuse to eat, and this necessitated a change in the daily amounts fed. In some cases all of the feed was not consumed and consequently the refuse had to be weighed at the close of the period and a separate analysis made. Such a procedure no doubt affected the final results to a slight extent.

The grain mixture used in period I consisted of 100 parts ground corn, 100 parts ground oats and 50 parts of wheat bran. In periods II and III the grain mixture was changed to one containing 10 parts of linseed oil meal in addition to the grain mixture used in period I. The animal received distilled water and sodium chloride throughout the entire three periods. The periods were separated in each case by a preliminary period of 8 to 10 days. The goats were completely adapted to their "new home." After the close of the experiment, if the doors were left open, they were frequently found in the crates ready for another trial.

The goats were milked and fed twice daily. A definite daily aliquot of urine and milk was composited for each period and the analyses were made upon these composite samples. The feces were collected daily and weighed after drying, and sampled after drying and grinding at the end of each period.

The methods of analysis were the same as those given in the Ohio Agricultural Experiment Station, Bulletin 363, 1922. All determinations were made in triplicate.

In presenting the data herein we have followed the most excellent form that has been established by a former investigator.

Discussion. The composition of the milk is recorded in table 1. The composition of the milk shows that goat's milk has a higher calcium and chlorine content than normal cow's milk.[2] This in general agrees with the data presented by Bosworth and Van Slyke (7).

The most important results of the experiment are recorded in tables 2 to 4. These tables exhibit the amount of each constituent in the food, milk, urine and feces and the balance between income and outgo. These tables show the effect, if any, of the addition of a mineral supplement to the ration, and the effect due to the change from dry to green hay, in case of milking goats, and the change from green to dry hay in case of dry goats. Goat 2 being in an earlier period of lactation than goat 1, shows a greater loss of calcium and phosphorus per kilogram of

[2] Unpublished data.

body weight than goat 1. The results of sulphur and nitrogen balances seem to indicate that possibly there is a close relationship between these two elements, for a positive balance of sulphur indicates a positive or low negative balance of nitrogen. In period II there are evidences that the precipitated bone flour favorably influenced the balance of calcium and phosphorus, although the increase in the protein of the ration and the difference in the period of lactation may have been an influencing factor. Both of the milking goats in period II gave a positive balance of sodium and a negative balance of potassium, while the two dry goats gave a positive balance of both sodium and potassium.

TABLE 1

Composition of milk (per cent)

GOAT NUMBER	MOISTURE	NITROGEN	PROTEIN (N × 6.38)	ETHER EXTRACT	ASH	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS
Period I												
1	87.38	0.465	2.967	4.17	0.75	0.0384	0.1794	0.1152	0.0122	0.0284	0.1736	0.0854
2	88.70	0.446	2.845	2.83	0.77	0.0487	0.1776	0.1258	0.0114	0.0288	0.1662	0.0944
Period II												
5	85.54	0.504	3.216	5.38	0.82	0.0428	0.1615	0.1676	0.0131	0.0333	0.1344	0.1028
2	88.61	0.436	2.782	3.06	0.77	0.0420	0.1814	0.1243	0.0121	0.0276	0.1713	0.0989
Period III												
5	86.96	0.415	2.648	4.74	0.80	0.0424	0.1597	0.1431	0.0148	0.0283	0.1485	0.0935
2	87.55	0.475	3.031	3.87	0.79	0.0391	0.1798	0.1191	0.0139	0.0301	0.1577	0.1100

In comparing the calcium and phosphorus balances of goats 5 and 2 in periods II and III, one finds a gradual decrease in the amount of negative calcium and phosphorus balances in grams per kilogram of body weight as the period of lactation advances until they all become or probably will become positive. The balance of phosphorus becomes positive at an earlier period of lactation than the calcium balance becomes positive. These very outstanding differences may be due to the change from dry to green feed and therefore to a vitamin which assists calcium assimilation, but we are inclined to believe that it is partly due to the increase in the length of the period of lactation and a difference in digestibility of the rations. The influence of the former factor would be more apparent with the goat than with the cow, since its period of lactation is shorter than that of the cow.

TABLE 2

Period I: balance of minerals and nitrogen (grams)

GOAT NUMBER	AVERAGE DAILY MILK YIELD	DISTINGUISHING FEATURE OF RATIONS		SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
	grams		FOOD	45.38	102.26	13.52	20.62	12.45	72.17	18.83	136.73
			MILK	2.14	9.97	6.40	0.68	1.58	9.65	4.75	25.86
			URINE	21.17	85.03	0.75	8.80	7.47	42.07	0.43	95.38
			FECES	10.06	19.98	17.34	13.63	6.62	0.94	36.39	51.94
			BALANCE	+12.01	−12.72	−10.97	−2.48	−3.22	+19.51	−22.74	−36.45
1	556	Basal ration (dry hay)									
			FOOD	59.12	239.90	32.06	46.10	26.88	95.95	40.78	297.07
			MILK	9.67	35.28	24.99	2.27	5.72	33.02	18.75	88.52
			URINE	36.59	160.31	1.16	13.41	8.37	55.13	1.82	110.30
			FECES	6.50	44.31	31.80	30.41	12.78	2.75	54.61	106.34
			BALANCE	+6.36	±0.00	−25.89	+0.01	+0.01	+5.05	−34.40	−8.09
2	1656	Basal ration (dry hay)									
			FOOD	10.24	27.06	4.16	4.79	3.26	17.17	3.85	35.69
			MILK	2.37	30.35	0.50	1.79	4.03	10.84	0.20	64.09
			URINE	2.86	3.55	8.41	3.44	1.75	0.27	9.66	11.77
			FECES	+5.01	−6.84	−4.75	−0.44	−2.52	+6.06	−6.01	−40.17
			BALANCE								
3	Dry	Basal ration (green hay)									
			FOOD	45.87	66.12	8.83	15.76	10.63	73.85	15.63	111.54
			MILK	36.06	58.18	0.68	6.86	5.82	74.52	3.99	81.70
			URINE	9.33	9.95	10.69	9.00	4.13	1.14	22.60	28.03
			FECES	+0.48	−2.01	−2.54	−0.10	+0.68	−1.81	−10.96	+1.81
			BALANCE								
4	Dry	Basal ration (green hay)									

TABLE 3

Period II: Balance of minerals and nitrogen (grams)

GOAT NUMBER	AVERAGE DAILY MILK YIELD	DISTINGUISHING FEATURE OF RATIONS		SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
	grams										
5	1597	Basal ration plus bone flour (dry hay)	FOOD	51.72	183.10	58.67	42.30	22.10	87.89	69.40	241.40
			MILK	7.52	28.37	29.44	2.30	5.85	23.61	18.06	88.54
			URINE	30.95	124.97	2.10	13.66	6.83	64.21	0.29	93.62
			FECES	6.36	31.38	54.52	29.14	9.81	1.42	64.54	76.86
			BALANCE	+6.89	−1.62	−27.39	−2.80	−0.39	−1.35	−13.49	−17.62
2	1201	Basal ration plus bone flour (dry hay)	FOOD	67.75	258.44	82.37	60.11	31.59	115.98	99.04	345.12
			MILK	7.56	32.66	22.38	2.19	4.97	30.85	17.81	78.49
			URINE	44.20	177.19	0.98	15.29	9.94	75.80	4.61	161.76
			FECES	7.75	51.63	61.35	34.42	16.50	3.24	73.44	108.17
			BALANCE	+8.24	−3.04	−2.34	+8.21	+0.18	+6.09	+3.18	−3.30
1	Dry	Basal ration plus bone flour (green hay)	FOOD	52.24	109.30	48.44	27.14	15.00	89.64	50.14	154.06
			MILK	35.98	95.62	0.79	14.22	8.29	82.36	2.48	117.88
			URINE	9.84	11.39	36.46	15.46	6.41	1.67	38.26	41.84
			FECES	+6.42	−2.29	+11.19	−2.54	+0.30	+5.61	+9.40	−5.66
			BALANCE								
4	Dry	Basal ration plus bone flour (green hay)	FOOD	53.10	124.84	55.99	32.70	18.67	91.56	63.92	195.67
			MILK	35.81	107.36	1.47	16.66	9.95	88.10	0.44	142.55
			URINE	13.07	15.82	50.31	17.23	7.19	1.06	60.23	50.86
			FECES	+4.22	+1.66	+4.21	−1.19	+1.73	+2.40	+3.25	+2.26
			BALANCE								

TABLE 4

Period III: Balance of minerals and nitrogen (grams)

GOAT NUMBER	AVERAGE DAILY MILK YIELD	DISTINGUISHING FEATURE OF RATIONS		SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
	grams		FOOD	58.28	215.09	75.28	45.03	27.10	107.63	94.06	296.79
			MILK	8.42	31.73	28.43	2.95	5.62	29.50	18.57	82.42
5	1325	Basal ration plus bone flour (green hay)	URINE	38.00	130.64	3.16	17.59	10.16	70.15	0.39	146.85
			FECES	5.75	31.87	62.30	25.92	9.90	1.16	77.34	75.27
			BALANCE	+6.11	+20.85	−18.61	−1.43	+1.42	+6.82	−2.24	−7.75
			FOOD	58.60	250.76	79.77	53.10	32.33	110.35	107.61	355.11
			MILK	5.81	26.72	17.70	2.07	4.47	23.43	16.35	70.54
2	991	Basal ration plus bone flour (green hay)	URINE	41.11	161.10	0.58	16.37	13.27	69.90	10.59	181.04
			FECES	5.26	44.54	54.11	32.96	11.11	2.22	67.52	86.54
			BALANCE	+6.42	+18.40	+7.38	+1.70	+3.48	+14.80	+13.15	+16.99
			FOOD	36.02	139.70	40.95	26.52	11.45	62.50	36.19	138.45
			MILK	30.35	131.55	0.60	9.40	7.43	71.91	1.84	114.96
1	Dry	Basal ration plus bone flour (dry hay)	URINE	6.57	7.17	34.20	14.94	5.88	1.24	30.67	40.90
			FECES	−0.90	+0.98	+6.15	+2.18	−1.86	−10.65	+3.68	−17.41
			BALANCE								
			FOOD	14.48	71.57	21.29	14.21	6.83	23.00	21.70	83.05
			MILK	11.37	62.34	0.51	6.80	5.93	29.58	2.41	92.05
4	Dry	Basal ration plus bone flour (dry hay)	URINE	4.01	5.75	21.52	7.81	3.46	1.26	18.22	24.13
			FECES	−0.90	+3.48	−0.74	−0.40	−2.56	−7.84	+1.07	−33.13
			BALANCE								

TABLE 5

Period I: Distribution of outgo of elements (per cent)

GOAT NUMBER	DISTINGUISHING FEATURE OF RATIONS	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
		MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES
1	Dry feed	6.4	8.7	26.2	2.9	10.1	18.3	11.5	14.9
		63.5	73.9	3.0	38.1	47.7	79.9	1.0	55.1
		30.1	17.4	70.8	59.0	42.2	1.8	87.5	30.0
2	Dry feed	18.3	14.7	43.1	4.9	21.3	36.3	24.9	29.0
		69.3	66.8	2.0	29.1	31.1	60.7	2.4	36.1
		12.3	18.5	54.9	66.0	47.6	3.0	72.6	34.9
3	Green feed								
		45.3	89.5	5.7	34.2	69.8	97.6	2.0	84.5
		54.7	10.5	94.3	65.8	30.2	2.4	98.0	15.5
4	Green feed								
		79.4	85.4	6.0	43.3	58.5	98.5	15.0	74.5
		20.6	14.6	94.0	56.7	41.5	1.5	85.0	25.5

TABLE 6

Period II: Distribution of outgo of elements (per cent)

GOAT NUMBER	DISTINGUISHING FEATURE OF RATIONS	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
		MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES	MILK URINE FECES
5	Dry feed plus calcium supplement	16.8	15.4	34.2	5.1	26.0	26.5	21.8	34.2
		69.0	67.7	2.4	30.3	30.4	71.9	0.3	36.1
		14.2	16.9	63.4	64.6	43.6	1.6	77.9	29.7
2	Dry feed plus calcium supplement	12.7	12.5	26.4	4.2	15.8	28.1	18.6	22.5
		74.3	67.8	1.2	29.5	31.7	69.0	4.8	46.4
		13.0	19.7	72.4	66.3	52.5	2.9	76.6	31.1
1	Green feed plus calcium supplement	78.5	89.4	2.1	47.9	56.4	98.0	6.1	73.8
		21.5	10.6	97.9	52.1	43.6	2.0	93.9	26.2
4	Green feed plus calcium supplement	73.3	87.2	2.8	49.2	58.1	98.8	0.7	73.7
		26.7	12.8	97.2	50.8	41.9	1.2	99.3	26.3

TABLE 7

Period III: Distribution of outgo of elements (per cent)

GOAT NUMBER	DISTINGUISHING FEATURE OF RATIONS		SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	SULFUR	CHLORINE	PHOSPHORUS	NITROGEN
5	Green feed plus calcium supplement	MILK	16.1	16.3	30.3	6.3	21.9	29.3	19.3	27.1
		URINE	72.8	67.3	3.4	37.9	39.6	69.6	0.4	48.2
		FECES	11.1	16.4	66.3	55.8	38.5	1.1	80.3	24.7
2	Green feed plus calcium supplement	MILK	11.1	11.5	24.4	4.0	15.5	24.5	17.3	20.9
		URINE	78.8	69.3	0.8	31.8	46.0	73.1	11.2	53.5
		FECES	10.1	19.2	74.8	64.2	38.5	2.4	71.5	25.6
1	Dry feed plus calcium supplement	MILK	82.2	94.8	1.7	38.6	55.8	98.3	5.7	73.7
		URINE								
		FECES	17.8	5.2	98.3	61.4	44.2	1.7	94.3	26.3
4	Dry feed plus calcium supplement	MILK	73.9	91.6	2.3	46.5	63.1	95.9	11.7	79.2
		URINE								
		FECES	26.1	8.4	97.7	53.5	36.9	4.1	88.3	20.8

The distribution of the outgo of the different elements is recorded in tables 5 to 7. Of the outgoing sodium, a goat in the early period of lactation gave the largest per cent in the milk (maximum 18.3), and the percentage decreased as the period of lactation advanced, while the urinary sodium increased as the period of lactation advanced. The

TABLE 8

Coefficients of digestibility of rations

GOAT NUMBER	DISTINGUISHING FEATURE OF RATIONS	YIELD OF MILK PER DAY	PROTEIN	NITROGEN FREE EXTRACT	CRUDE FIBER	ETHER EXTRACT	NUTRITIVE RATIO OF RATIONS
			Period 1				
		grams					
1	Dry feed	556	62.01	74.90	59.61	80.66	1:7.8
2	Dry feed	1656	64.20	74.60	62.01	83.69	1:7.6
3	Green feed	Dry	67.04	74.27	67.30	74.75	1:7.5
4	Green feed	Dry	74.87	81.79	59.56	84.34	1:7.1
			Period II				
5	Dry feed plus calcium supplement	1597	68.16	72.40	68.17	81.27	1:7.0
2	Dry feed plus calcium supplement	1201	68.66	74.15	51.56	68.40	1:6.6
1	Green feed plus calcium supplement	Dry	72.84	77.59	56.70	71.89	1:6.5
4	Green feed plus calcium supplement	Dry	74.01	78.10	51.23	69.29	1:6.2
			Period III				
5	Green feed plus calcium supplement	1324.4	74.64	78.97	50.90	75.65	1:6.0
2	Green feed plus calcium supplement	991	75.63	78.79	52.99	78.86	1:6.3
1	Dry feed plus calcium supplement	Dry	70.46	70.70	64.11	65.42	1:6.5
4	Dry feed plus calcium supplement	Dry	70.95	73.11	60.71	66.96	1:6.6

fecal sodium was higher in the case of the dry goats than with the milking goats, while the opposite is true of the fecal potassium, thus showing a physiological antagonism.

The outgo of the calcium in the milk reached its maximum (43.1 per cent) with goat 2 in the early period of lactation (period I). The maximum phosphorus outgo in the milk (24.9 per cent) was with this same goat in the same period. The variation of the calcium in the urine was between 0.8 and 6.0 per cent, while the fecal calcium varied from

54.9 to 98.3 per cent, the highest amount in each case being with dry goats. In the case of dry goats the magnesium seems to have been deflected from what would have been in the milk to the urine, while the calcium and phosphorus were deflected in the feces. The elimination of sulphur and nitrogen in the milk reached a maximum with the freshest goat, and decreased as the period of lactation advanced. The elimination of chlorine in the milk reached a maximum of 36.3 per cent; in the urine it varied from 60.7 to 98.8 per cent, while it varied in the feces from 1.1 to 3.0 per cent. The sulphur, chlorine and nitrogen which were in the milk are deflected to the urine when the animal becomes dry.

The coefficient of the digestibility of the rations are recorded in table 8. One observes that as a rule the average digestibility of the proteins, carbohydrates and ether extracts is increased in changing from a dry ration to a ration containing green roughage, and decreases in changing from a ration containing green roughage to a dry ration. The reverse is true in the case of crude fiber. Henry and Morrison[3] state that heating a hay until browned caused an increased digestibility of the crude fiber, but decreased the digestibility of the crude protein and nitrogen free extract (carbohydrates). In the case of green roughage the microörganisms in the digestive tract may act first upon the more readily available cell contents (protein, carbohydrates and mineral matter), which have been liberated by mastication—thus satisfying their food requirements and therefore do not attack the crude fiber. Possibly the above is an explanation of our results. These findings seem to be contrary to the statements in the literature, that there is practically no difference in the digestibility of green and dry feed. It is true that the data in the literature refer to experiments carried out with green and dry hay alone, without any grain supplement, and under such conditions there may be no difference in the digestibility but we know that there is a change (physical) in the cell wall and cell content when the plant dries and as a result one would naturally think that this change would affect digestibility. Unfortunately we have no evidence that would indicate to us whether it was the grain or the green roughage that was better digested, but we believe it was the latter. We are inclined to believe then that the effect of green feed in causing the difference in the assimilation of calcium over that of dry feed is due to the fact, other than the vitamin theory, that the calcium in the cells of green plants and hay not exposed to an excessive drying is more highly dispersed and therefore better digested and assimilated than the calcium in dry hay. The vitamin theory and the theory here presented may be

[3] *Feeds and Feeding*, 1922 edition.

one and the same theory. We have evidence, which has been reported (8), that tricalcium phosphate precipitated in a starch paste caused a positive balance in five out of six complete accountings of calcium, with two milking goats on a ration of grain and dry timothy hay. It is true that the results in this case may be due to the lateness in the period of lactation (fourth and fifth months), yet the goats were giving from 750 to 850 grams milk per day, and continued to give a fair quantity of milk for four or five months longer, which would indicate that they were in about the middle of their period of lactation.

The nutritive ratio in periods II and III varied to some extent, but this was due to the individuality of the animal. The nutritive ratio in period I was, no doubt, too wide for normal milk production and an attempt was made with some success to change this to a more narrow one in periods II and III. Our results should be interpreted with this in mind.

In general the mineral metabolism of the goat is very similar to that of the cow. The goat is a very good experimental animal, and since the feed consumption is not so large and the excrements are not so bulky we believe that more excellent results can be secured, in such tests with goats than with cows.

SUMMARY

The calcium and chlorine content of goat's milk is higher than that of cow's milk.

In the early period of lactation there is a loss of calcium and phosphorus from the body of lactating goats irrespective of the nature of the feed. As the period advances these losses are decreased until a certain time, when the balances becomes positive. The time at which the phosphorus balance becomes positive is not necessarily the same time at which the calcium balance becomes positive.

A goat in the early period of lactation gave the largest per cent of sodium in the milk and this decreased as the period advanced. A high fecal sodium was associated with a low fecal potassium and vice versa.

In the case of dry goats the magnesium seems to have been deflected from the milk to the urine, while the calcium and phosphorus were deflected to the feces.

The sulphur, chlorine and nitrogen which are in the milk are deflected to the urine when the animal becomes dry.

The coefficients of digestibility of the protein, nitrogen-free extract (carbohydrates) and ether extract of green feed are higher than those of dry feed, while the crude fiber is lower. This suggests to us the possibility that this is the real cause of the difference in the assimilation of calcium.

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BIBLIOGRAPHY

(1) FORBES, BEEGLE, FRITZ, MORGAN AND RHUE: Ohio Agric. Exper. Sta., Bull. 295, 1916; Bull. 308, 1917; FORBES, HALVERSON AND MORGAN: Ohio Agric. Exper. Sta., Bull. 330, 1918; FORBES, HUNT, SCHULTZ, WINTER AND REMLER: Ohio Agric. Exper. Sta., Bull. 363, 1922.

(2) HART, MCCOLLUM AND HUMPHREY: Wisconsin Agric. Exper. Sta. Research Bull. 5, 1908.

(3) MEIGS, BLATHERWICK AND CARY: Journ. Biol. Chem., 1919, xl, 469.

(4) HART, STEENBOCK AND HOPPERT: Journ. Biol. Chem., 1921, xlviii, 33; Science, 1920, lii, 318.

(5) HART, STEENBOCK, HOPPERT, BETHKE AND HUMPHREY: Journ. Biol. Chem., 1922, liii, 21.

(6) HART, STEENBOCK, HOPPERT, BETHKE AND HUMPHREY: Journ. Biol. Chem., 1922, liv, 75.

(7) BOSWORTH AND VAN SLYKE: N. Y. (Geneva) Exper. Sta., Tech. Bull. 46, 1915.

(8) HUNT, WINTER AND MILLER: Journ. Biol. Chem., 1923, lv, 739.

THE EFFECT OF DIURESIS ON EXCRETION OF URIC ACID

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Sodium chloride and sodium sulphate diuresis have been thoroughly studied with regard to many of their effects on both blood and urine. Of the urinary constituents so studied, however, uric acid has received little attention. It has been the purpose of this investigation primarily to discover as many facts as possible concerning the effect of diuresis on this substance, and to relate these facts with the known behavior of other substances.

Salt diuresis was made the subject of extensive work by Magnus (1), Sollmann (2), Cushny (3) and others. In general, there is agreement that sulphates and chlorides represent two different groups, of which the former is excreted with more difficulty. Upon being injected intravenously they act upon the kidney by causing hydremia. To some extent both leave the blood stream for the tissues at once, although chlorides may be much more efficient in this. As a result, excretion of urine will begin more promptly after sulphate injection. At the height of the reaction, however, the hydremia is not greater than in chloride diuresis. A large proportion of the chloride solution injected may remain in the tissues at the end of the diuresis, resulting in a smaller proportional excretion of urine. One feature which is common to both is the invariable reduction in concentration of plasma proteins.

Concerning the mechanical factors, Lamy and Mayer (4) state that large injections increase, while small injections do not increase the blood flow through the kidney. Barcroft and Brodie (5) and Barcroft and Straub (6) found that the blood pressure and blood flow, as compared with controls, were little affected. In general it may be said that the mechanical factors do not suffice to explain the increased excretion but may play a part. The changes are, however, not significantly different for the two forms of diuresis.

The effect on the urinary constituents in either form of diuresis may be summed up as follows: *a*, The "non-threshold" substances, urea, sulphates, etc., will increase in total quantity, the maximum excretion taking place at the beginning of diuresis. On the other hand, the

percentage will fall below normal to rise again as the amount of urine lessens. *b*, The "threshold" substances, chlorides, sugar, etc., will also show an increased total excretion at the beginning of diuresis, but the percentage will vary according to the previous condition of the urine. Where the percentage has been low before injection it will be increased, and vice versa. Even under the injection of chlorides the maximum percentage will be reached during the early stages, with a progressive fall as the urine flow abates. The injection of either salt will apparently to some extent replace the other, making increased elimination possible. *c*, Cushny (7) places uric acid and potassium in a class by themselves, the behavior of which is said to be intermediate between the foregoing two classes.

Another interesting aspect of the subject was treated by Barcroft and collaborators (5), in their consideration of oxygen consumption. Briefly, they found that the diuresis following injection of sodium chloride or Ringer's solution causes no elevation of the oxygen consumption, while under sodium sulphate and urea it does increase. However, when they came to interpret their results they found that the work of concentration (in sulphate diuresis) as calculated from the depression of freezing point of blood and urine at various intervals, accounted for only a small fraction of the energy represented by the increase in oxygen consumed; nor did these two bear any relation to each other in sense or degree. Increase in the rate of excretion was always accompanied by increased oxygen consumption, but the two showed no uniform ratio in different periods of the same experiment and, in different animals, the same figure for oxygen consumed, per cubic centimeter of urine excreted, was only approached during the height of diuresis. They did not unfortunately study depression of the freezing point in chloride experiments. As a measure of kidney work, then, oxygen consumption can not be taken as a reliable index.

Before considering the work done on the excretion of uric acid itself, it may be well to refer to a possibility which the authors considered in undertaking this investigation. If, as is generally now conceded, endogenous uric acid represents the catabolic phase of nuclear activity, why should not work on the part of a highly cellular organ be represented by increased production of this substance? Prolonged diuresis, especially of a variety causing active work, as seems to be the case after sulphate injection, should gradually increase the store of uric acid available for excretion and result in a curve differing from both the other classes of urinary constituents already considered. Obvious technical difficulties preclude securing direct evidence bearing on this question. Under the condition of the present experiments, the data secured do not indicate such a production of uric acid.

Burian and Schur (8), in the course of their work, observed that intravenous injection in dogs resulted in an increase in the hourly excretion of uric acid. This increase, which was most marked during the first hour or two, exceeded the increase in total urinary nitrogen elimination. There occurred a compensatory fall during later hours, and a further experiment showed that the 24-hour uric acid output was not increased. In man, the taking of diuretics by mouth did not cause any increase either in the hourly or 24-hour output. They explained this on the basis of a disturbance in the rate of the normal destruction of uric acid in the body of the dog during diuresis, probably due to circulatory changes; and conversely not due to an increased production of this substance.

Robertson (9) worked with hens and one dog, and concluded that under some conditions uric acid excretion may vary independently of the urine flow. He made no analysis of other urinary constituents, and admitted himself that his results were unsatisfactory.

Methods. The work here reported was done on rats and rabbits. In the case of the former, urine was collected through funnels placed under wire cages, and caught in volumetric flasks. The volume was made up with wash water. The procedure has been described by Ordway and Morris (10). The animals were fed throughout the experimental period on a thin soup of cracker crumbs and warm water. Injection was into the saphenous vein by Roth's technic (11), the solution being allowed to run in by gravity. All rats were etherized for injection.

Our first three rabbits were decerebrated under ether by trephining a small hole near the occipital suture and introducing a hemostat. After pinching off the brain stem just anterior to the bony tentorium, the hemostat was left in place. A glass tube with sufficient flare at the proximal end to cover the openings of both ureters was introduced into the bladder, and ligatured into place. Injection was by gravity into the external jugular vein. Subsequent animals were tied down without being anesthetized, and a fine catheter passed through the urethra into the bladder. The bladder was then washed several times with normal saline, until the wash water returned clear. Injection was into the marginal ear vein.

The determination of uric acid was done by the method of Morris and Macleod (12). Total nitrogen, urea and creatinine were determined by adaptations of the methods of Folin and Wu (13) for blood analysis. An attempt was made to estimate allantoin by the Wiechowski method, but this proved unsatisfactory on account of the small amount of urine available and the extreme dilution. Chlorides were ascertained by the Whitehorn (14) method. The freezing point determinations were made in a Beckmann-Heidenhain apparatus.

EXPERIMENTAL. *Group 1.* Table 1 shows the effect on the 24-hour output of uric acid and total urinary nitrogen by a single injection of a salt. The animals were injected throughout with from 10 cc. to 15 cc.

TABLE I

Effect of intravenous injection of salt solutions on 24-hour total nitrogen and uric acid excretion

First series

	RAT A (170 GRAMS)		RAT B (158 GRAMS)		RAT C (172 GRAMS)		RAT D (255 GRAMS)		RAT X (310 GRAMS)		RAT Y (165 GRAMS)	
	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Foreperiod												
1st day	78.8	1.20	84.0	0.98	63.8	0.63						
2nd day	78.0	1.00	102.0	1.12	82.5	0.78	124.5	1.24	129.0	2.22	139.5	2.18
3rd day	73.5	1.34	111.0	1.22	84.8	0.63	136.5	1.02	130.5	2.22	102.0	1.38
Average day	76.8	1.18	99.0	1.11	77.0	0.68	130.5	1.13	130.0	2.22	121.0	1.78
Injection	4 per cent Na_2SO_4		4 per cent Na_2SO_4		1.7 per cent NaCl		1.7 per cent NaCl		0.9 per cent NaCl		0.9 per cent NaCl	
4th day	76.5	1.96	124.5	1.66	91.5	1.01	138.0	1.60	141.0	2.36	138.0	2.50
Increase	0%	66%	26%	50%	19%	49%	6%	42%	9%	6%	14%	40%
5th day	85.5	1.38	102.0	1.30	96.8	0.86	120.0	1.14	153.0	2.32	81.0	1.32

Second series

	RAT A (157 GRAMS)		RAT B (150 GRAMS)		RAT C (165 GRAMS)		RAT D (244 GRAMS)	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Foreperiod								
1st day	115.5	0.96	92.3	1.09	73.5	0.65	132.0	0.88
2nd day	99.8	1.00	108.0	0.55	103.5	0.71	111.0	1.09
3rd day	114.0	0.95	112.5	0.98	123.0	0.83	147.0	1.18
Average day	109.8	0.97	104.3	0.87	100.0	0.73	130.0	1.05
Injection	1.7 per cent NaCl		1.7 per cent NaCl		4 per cent Na_2SO_4		4 per cent Na_2SO_4	
4th day	96.8	0.99	97.5	1.18	114.0	1.15	162.0	1.69
Increase	−12%	2%	−6%	36%	14%	58%	25%	61%
5th day	122.3	1.14	99.8	0.99	114.0	0.71	127.5	0.91

according to weight. Animals A, B, C and D were used again after an interval of one to three weeks for injection with the salt to which they had not originally been subjected. The following relationships are believed to be interesting: Uric acid makes an average gain of 52 per

cent (exclusive of animal A, second series) over the average of the three preceding days, while total nitrogen gains but 12 per cent. When the animals are grouped according to the substance injected, the sulphate animals gain 59 per cent and 16 per cent for uric acid and total nitrogen respectively, while the chloride animals make 42 per cent and 6 per cent gains. No constant or significant effect on the output of either substance on the day following the 24-hour test period can be seen, except in the above mentioned case of animal A, second series, where there is evidently a delayed reaction. The 24-hour uric acid output for the sulphate animals when figured per 100 grams body weight was 0.87 mgm.; the chloride animals excreted 0.65 mgm.

TABLE 2

Hourly excretion following single intravenous injection of salt solutions

Injection.....	1.7 per cent NaCl		4 per cent Na_2SO_4		0.9 per cent NaCl				2 per cent Na_2SO_4			
	Rat E (210 gms.)	Rat F (270 gms.)	Rat G (260 gms.)	Rat H (195 gms.)	Rat I (162 gms.)		Rat J (190 gms.)		Rat K (175 gms.)		Rat L (218 gms.)	
TIME AFTER INJECTION	Uric acid	Uric acid	Uric acid	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid	Total nitrogen	Uric acid
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1st hour...	0.11	0.19	0.20	0.19	9.3	0.25	13.5	0.32	7.5	0.31	10.2	0.20
2nd hour...	0.13	0.11	0.20	0.13					5.5	0.15	12.2	0.20
3rd hour...	0.14	0.12	0.07	0.05	9.8	0.10	9.4	0.12	6.4	0.15	4.3	0.08
4th hour...	0.11	0.11	0.04	0.04	10.0	0.13	7.4	0.08				
5th to 24th hour.....	2.14	2.21	2.17	1.05	73.5	0.82	81.0	0.84	73.5	0.90	11.5	1.46

Group 2. In order to ascertain whether the 24-hour method of collection masked any significant differences between the two diuretics, hourly collections were made use of in a second group (table 2). It is evident that sulphate, whether isotonic or hypertonic, causes a large secretion of uric acid in the first hour or two with a compensatory drop later. This is to be contrasted with the tendency toward a more even level in the output of the chloride animals. When the 24-hour uric acid output per 100 grams body weight is compared on the basis of diuretics used, as in the previous group, sulphates show 0.88 mgm., chlorides 0.95 mgm. However, when the animals are grouped according to the concentration of the injected fluid, hypertonic injections produce 1.01 mgm., isotonic injections 0.81 mgm.

Total nitrogen, where determined in the chloride animals, seems to rise to its maximum in a later period than in the sulphate animals. Creatinine was determined throughout this series but the results seem to have questionable value, on account of the difficulty of obtaining accurate color comparisons in so dilute specimens.

Group 3. In view of the fact that the injection of diuretics apparently increased the 24-hour output of uric acid, it was decided to investigate the effect of repeated injections (table 3). In this group the urea was determined, and it is seen in a general way to be similar in its excretion to uric acid. An interesting feature is the delayed reaction occurring after the third injection in animals M, N, O, P.

TABLE 3

Effect of repeated injections of salt solutions on hourly excretion

	RAT M (160 GRAMS)		RAT N (185 GRAMS)		RAT O (230 GRAMS)			RAT P (215 GRAMS)			RAT R (200 GRAMS)		
	Urea nitrogen	Uric acid	Urea nitrogen	Uric acid	Urea nitrogen	Uric acid	Chloride	Urea nitrogen	Uric acid	Chloride	Urea nitrogen	Uric acid	Chloride
Injection	2 per cent Na_2SO_4		2 per cent Na_2SO_4		0.9 per cent NaCl			0.9 per cent NaCl			2 per cent Na_2SO_4		
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1st hour	2.9	0.25	3.4	0.24	12.7	0.21	5.0	1.9	0.03	1.7	3.5	0.34	0.4
2nd hour	3.0	0.12	4.2	0.16	7.8	0.16	7.8	10.9	0.10	15.3	3.7	0.18	0.1
Injection	2 per cent Na_2SO_4		2 per cent Na_2SO_4		0.9 per cent NaCl			0.9 per cent NaCl			2 per cent Na_2SO_4		
3rd hour	3.1	0.18	3.1	0.09	13.5	0.27	30.6	6.1	0.12	29.8	4.1	0.18	0.4
4th hour	1.7	0.06	8.6	0.18	6.5	0.14	14.3	9.3	0.11	33.8	3.5	0.12	0.1
Injection	2 per cent Na_2SO_4		2 per cent Na_2SO_4		0.9 per cent NaCl			0.9 per cent NaCl			2 per cent Na_2SO_4		
5th hour	2.4	0.09	2.8	0.07	4.0	0.08	16.0	5.1	0.09	22.5	5.6	0.15	1.8
6th hour	3.4	0.10	6.2	0.13	9.1	0.20	19.0	10.4	0.12	33.8	2.0	0.10	0.05
7th to 24th hour	53.0	1.44	82.0	2.27	108.0	2.7		76.0	2.1				

In line with the observation in the previous group, it is notable that the amount of uric acid excreted by the sulphate animals fell to lower levels with each injection, whereas chloride animals showed a tendency to maintain a more uniform excretion. That this is a compensatory process is shown by the fact that the total 6-hour excretion when figured on a basis of weight is practically the same for four out of five animals. Nor is there a significant difference in the 24-hour amount, being for sulphate and chloride, 1.55 mgm. and 1.44 mgm., respectively.

TABLE 4

Effect of intravenous injection of salt solution on 20-minute excretion of urinary constituents

	RABBIT 4 (2.6 KGM.)					RABBIT 5 (2.0 KGM.)					RABBIT 6 (2.6 KGM.)				
	Volume	F. P. dep.	Urea nitrogen	Uric acid	NaCl	Volume	F. P. dep.	Urea nitrogen	Uric acid	NaCl	Volume	F. P. dep.	Urea nitrogen	Uric acid	NaCl
Injection	200 cc. 2 per cent Na_2SO_4					200 cc. 0.9 per cent NaCl					200 cc. 0.9 per cent NaCl				
	cc.		*mgm.*	*mgm.*	*mgm.*	*cc.*		*mgm.*	*mgm.*	*mgm.*	*cc.*		*mgm.*	*mgm.*	*mgm.*
1st 20 min	47	0.50	32.9	4.82	182	44.5	0.48	26.3	0.81	180	14.0	0.66	22.4	0.77	99
2nd 20 min	65	0.47	27.3	2.60	220	25.0	0.47	11.3	0.34	101	2.8	0.85	17.8	0.73	58
3rd 20 min	39	0.53	20.7	2.15	106	30.0	0.52	18.3	0.61	134	5.2				
4th 20 min	12	0.51	7.9	0.73	22	2.0					21.0	0.44	26.0	0.90	132
5th 20 min	11	0.52	7.8	0.69	16	2.5	0.72	9.2	0.60	32	19.0	0.31	16.2	0.54	94
6th 20 min	12.5	0.49	10.0	0.71	18	3.0					19.0	0.24	11.4	0.34	85
Injection	20 cc. 10 per cent Na_2SO_4					20 cc. 4.5 per cent NaCl					20 cc. 10 per cent Na_2SO_4				
7th 20 min	52	0.55	22.4	1.01	172	24	0.61	19.9	0.73	115	49.0	0.44	26.5	0.81	247
8th 20 min	33	0.51	14.9	0.58	103	22	0.56	12.5	0.34	109	46.0	0.52	16.6	0.51	232
9th 20 min	18.5	0.54	10.9	0.50	44	12.5	0.64	10.0	0.34	70	17.5	0.67	11.2	0.48	92
10th 20 min	11	0.64	9.1	0.59	5.5	6.5	0.75	8.6	0.34	42	11.0	0.69	8.1	0.47	59
11th 20 min	9	0.67	10.3	0.65	1.5	2.5	0.94	8.5	0.36	36	9.0	0.73	7.6	0.54	46
12th 20 min	6	0.75	8.2	0.51	0.8	2.5					4.2	0.79	4.2	0.32	19

These amounts are, however, worthy of comparison with those obtained in the preceding two groups by single injection.

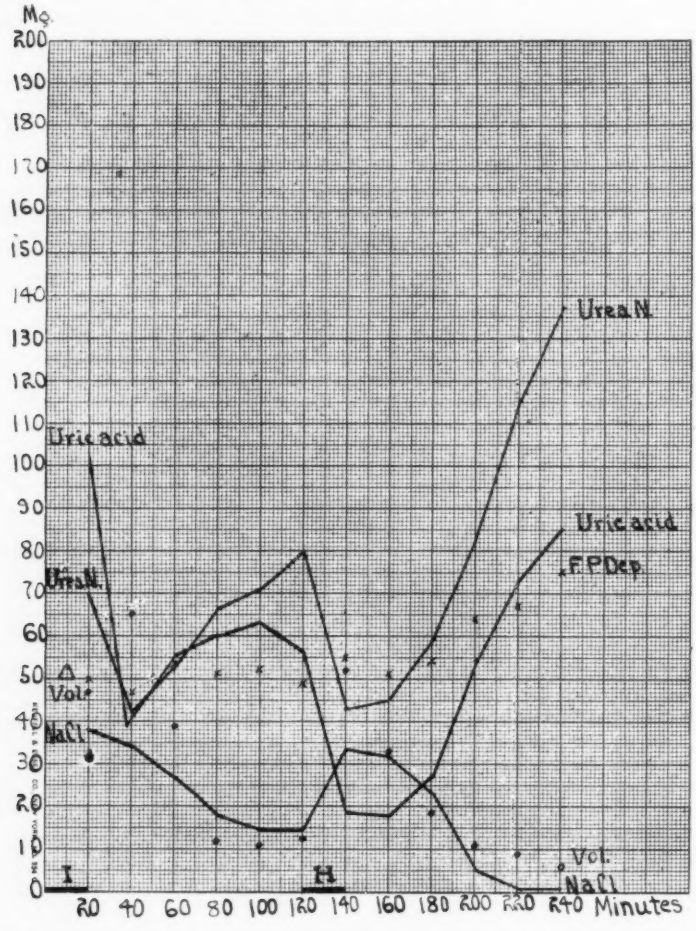

Chart 1. Rabbit 4. Isotonic sodium sulphate (200 cc.) injection, *I*, followed 2 hours later by (20 cc.) injection of hypertonic sodium sulphate, *H*. Uric acid values multiplied by 10, sodium chloride by $\frac{1}{10}$, to facilitate comparison with urea. Freezing point depresson and volume plotted arbitrarily for reference.

Group 4. In order to have sufficient material with which to make a more complete comparative study of the urinary constituents, and also to obtain if possible a clew to the relation which the process of urinary

concentration might bear to the behavior of uric acid, rabbits were selected for the final observations. Six experiments were carried

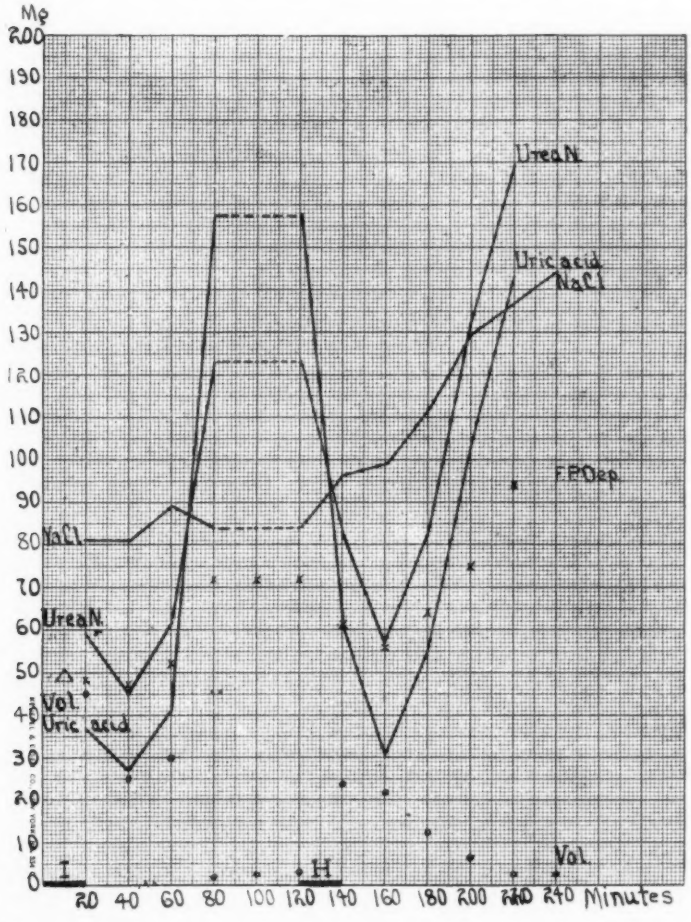

Chart 2. Rabbit 5. Isotonic sodium chloride (200 cc.) injection, *I*, followed 2 hours later by injection (20 cc.) of hypertonic sodium chloride, *H*. Uric acid values multiplied by10, sodium chloride by $_1{}^1{}_0$, to facilitate comparison with urea. Freezing point depression and volume plotted arbitrarily for reference.

through but, on account of uncontrollable factors, the first three animals, which were decerebrate, gave unsatisfactory results. As far as they go they bear out, however, the facts as here set forth. The three remain-

ing animals, although showing much less uniformity in total uric acid output than the rats, were individually consistent. The output in the

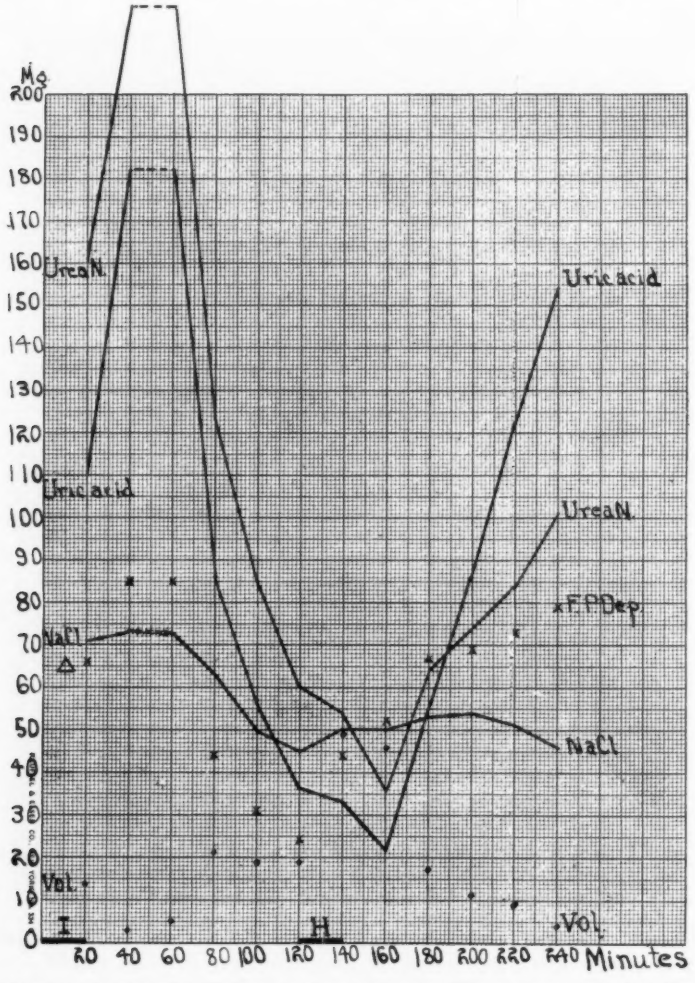

Chart 3. Rabbit 6. Isotonic sodium chloride (200 cc.) injection, *I*, followed 2 hours later by injection (20 cc.) of hypertonic sodium sulphate, *II*. Uric acid values multiplied by 10, sodium chloride by $\frac{1}{10}$, to facilitate comparison with urea. Freezing point depression and volume plotted arbitrarily for reference.

same animal for two hours following each of two consecutive injections is about equal for either salt and for different concentrations. In

general the volume (table 4) follows the total excretion of salts as exemplified by the chlorides, while urea and uric acid conform to the same general curve. In charts 1, 2, 3 the results are represented on a concentration basis (milligrams per 100 cubic centimeters). It is there apparent that uric acid follows very closely the curve of urea; both of these substances differing markedly from the chlorides.

Discussion. It is evident from consideration of the first three groups, that strenuous diuresis will increase the total uric acid output in 24 hours. It will moreover, increase in much greater proportion than the total urinary nitrogen. This apparent contradiction of the results of Burian and Schur is probably due to the greater magnitude of the diuresis. The even larger increase observed under repeated injections bears out such an explanation. It was obviously impossible to determine whether their hypothesis of the unbalancing of the uricolytic process is correct, since satisfactory allantoin determinations could not be made.

Concerning the effect of different classes of diuretics, it seems fair to conclude that their temporarily different effects are due to the manner in which they effect diuresis, and that these differences are compensated within less than 24 hours. That different concentrations of a salt affect uric acid excretion differently is probably to be ascribed to the greater diuretic effect produced by a hypertonic solution when given with an equal amount of liquid. The small volume given would explain the authors' failure to find anything different between the injection of isotonic and hypertonic solutions in the rabbit, where the latter was five times the concentration of the former, instead of twice as in the rats.

The excretion of uric acid apparently follows the same laws as that of urea; the findings presented here lend no support to the theory that it occupies an intermediate position. In some of the rats it appears at times that the initial excretion following a first injection was greater in proportion to its subsequent level than for other substances. This may be explained on the assumption that a larger amount of uric acid is circulating in the blood at the time of injection than at any later period; when this is disposed of, the amount supplied from endogenous sources is small.

As with urea, the output of uric acid is affected by the volume of urine excreted more than by any other factor; nor is there anything in its behavior which makes it necessary to call in data on freezing point depression to explain significant variations from the rule for so-called non-threshold substances.

SUMMARY

Uric acid excretion is increased to a similar degree by chloride and sulphate diuresis. There is no evidence of uric acid arising directly from increased kidney activity. The increased output is proportional to the volume of urine excreted. The variations in amount of uric acid excreted after isotonic and hypertonic salt injections indicate that its usual behavior is that of a non-threshold substance.

We wish to express our appreciation of helpful suggestions made by Dr. Torald Sollmann during this investigation.

BIBLIOGRAPHY

(1) Magnus: Arch. exper. Path. u. Pharm., 1901, xliv, 68, 396.
(2) Sollmann: Arch. exper. Path. u. Pharm., 1901, xlvi, 1.
(3) Cushny: Journ. Physiol., 1901, xxvii, 429.
(4) Lamy andMayer: Journ. de Physiol. et Path., 1906, viii, 258, 660.
(5) Barcroft and Brodie: Journ. Physiol., 1905, xxxiii, 52.
(6) Barcroft and Straub: Journ. Physiol. 1910, xli, 145.
(7) Cushny: The secretion of urine. London, 1917, 135.
(8) Burian and Schur: Pflüger's Arch., 1901, lxxxvii, 239.
(9) Robertson: This Journal, 1914, xxxiii, 324.
(10) Ordway and Morris: Journ. Med. Res., 1913, xxviii, 301.
(11) Roth: Public Health Reports, 1921, xxxvi, 661.
(12) Morris and Macleod: Journ. Biol. Chem., 1922, 1, 55.
(13) Folin and Wu: Journ. Biol. Chem., 1919, xxxviii. 81.
(14) Whitehorn: Journ. Biol. Chem., 1921, xlv, 449.

THE PRODUCTION OF HYPOGLYCEMIA IN EXPERIMENTAL DERANGEMENTS OF THE LIVER

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Experimental evidence has been adduced by Mann and Magath (1) to show that the liver plays an important rôle in the regulation of the normal concentration of the sugar in the blood. Following the total extirpation of the liver in dogs, a progressive decrease of the blood sugar occurs. No ill effects are observed until the blood sugar reaches about 0.06 per cent, when the first signs of muscular weakness are noted. Shortly thereafter and concomitant with a further decrease in the blood sugar level, the animal becomes perfectly flaccid and comatose, then muscular twitchings appear which become more and more severe, the animal usually dying in convulsions. Mann and Magath have demonstated the remarkable fact that, at the time of death, the blood sugar concentration is about 0.04 per cent. If glucose is injected at this stage, the animal may show prompt, though temporary, recovery from these symptoms.

Quite recently Williamson and Mann (2) performed a series of experiments with the object of determining whether the characteristic symptoms and changes in the blood which invariably follow total removal of the liver by surgery, would occur likewise after the administration of chloroform and phosphorus. Williamson and Mann report that the characteristic symptoms which follow hepatectomy were not observed after poisoning with these substances, although these intoxications frequently resulted fatally. Hypoglycemia occurred in but a small percentage of their experiments.

Hendrix and Sweet (3) have shown recently that glucose practically disappears from the urine of Eck's fistula pancreadectomized dogs. They attribute this to a diminished transformation of the sugar-forming amino acids into glucose and suggest that an important function of the liver is to synthesize glucose from amino acids. In support of this view, these authors point out that hypoglycemia develops when the liver is removed (4), or when it is severely injured by poisoning with phosphorus (5), hydrazine (6) and chloroform (unpublished data by Bodansky).

In a number of experiments it has been found that hypoglycemia occurs in severe chloroform poisoning just as in hydrazine and phosphorus intoxications, this effect being especially prominent in poorly nourished animals. The initial effect of severe liver damage produced by the administration of these compounds is usually hyperglycemia. As the carbohydrate store is depleted, the preliminary effect disappears and the blood sugar level falls to subnormal values. The following data were obtained in a case of chloroform poisoning by prolonged anesthesia. It is to be noted that on the fifth day of the experiment, the blood sugar concentration was definitely below normal.

1. Dog, male, weight 8.7 kgm.
   1st day: Blood sugar—0.099 per cent
   2nd day: Chloroform anesthesia for 2 hours
   3rd day: Chloroform anesthesia for 1 hour
   4th day: Chloroform anesthesia for 1 hour, 30 minutes
   5th day: Blood sugar—0.065 per cent
   6th day: Blood sugar—0.070 per cent
   9th day: Blood sugar—0.080 per cent

In a second experiment the subcutaneous injection of chloroform resulted at first in an increase in the blood sugar which was followed by hypoglycemia.

2. Dog, male weight 7.8 kgm.
   1st day: Blood sugar—0.096 per cent; two cubic centimeters of chloroform in olive oil injected subcutaneously
   2nd day: Blood sugar—0.121 per cent
   5th day: Blood sugar—0.125 per cent; 4 cc. of chloroform in olive oil injected subcutaneously
   6th day: Blood sugar—0.083 per cent; 3 cc. of chloroform in olive oil injected subcutaneously
   9th day: Blood sugar—0.094 per cent
   12th day: Blood sugar—0.067 per cent

Similar observations were made in a case of phosphorus poisoning, as shown by the following data.

3. Dog, female, weight 13.0 kgm.
   1st day: Blood sugar—0.096 per cent
   2nd day: 5 mgm. phosphorus in olive oil injected subcutaneously
   5th day: Blood sugar—0.108 per cent; 5 mgm. phosphorus in olive oil injected subcutaneously
   8th day: Blood sugar—0.068 per cent

As pointed out by Williamson and Mann, functional damage to the liver is probably not the primary cause of death in severe intoxications with chloroform and phosphorus. We believe, however, that under certain conditions effects similar to those described in the case of com-

pletely hepatectomized dogs can be brought on by the administration of large doses of chloroform, phosphorus or hydrazine to animals whose livers had been extensively damaged by previous injections of the same compounds.

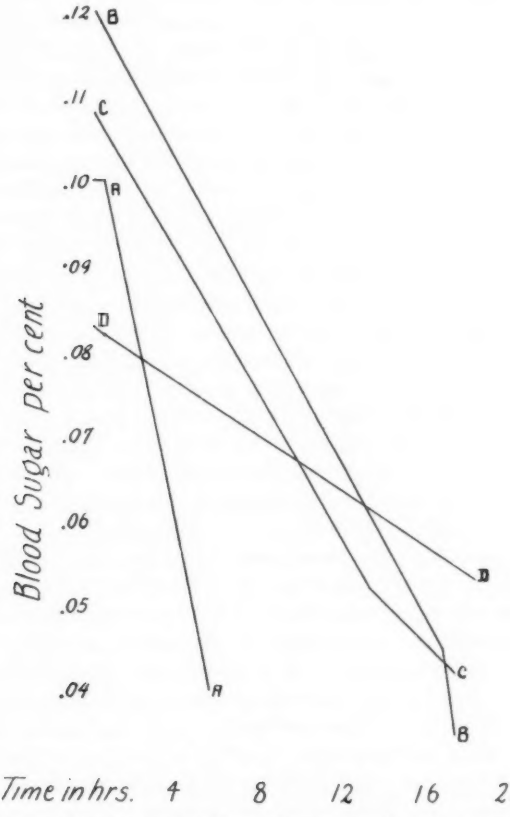

Fig. 1. Curves showing terminal hypoglycemia produced, *A*, on removal of the liver—average obtained on 13 dogs from the work of Mann and Magath; *B*, in phosphorus poisoning; *C*, in hydrazine poisoning; *D*, in chloroform poisoning.

Severe chloroform poisoning was produced in a dog by the subcutaneous administration of 4 cc. of chloroform in olive oil. Much improvement was noted on the tenth day of the experiment when a second injection was administered. This resulted fatally, death being preceded by a relatively pronounced hypoglycemia (0.053 per cent). Microscopic examination of the liver tissue showed hemorrhagic areas,

marked nuclear and protoplasmic degeneration together with extensive necrosis in certain areas.

Extensive liver injury was produced in another dog. This animal died four days after the subcutaneous injection of 10 mgm. of phosphorus in olive oil. Three specimens of blood were collected shortly before death. At 8:35 a.m. the blood sugar concentration was 0.045 per cent. Twenty minutes later it was 0.040 per cent. At 9:25, or 15 minutes before death, the blood sugar had fallen to 0.035 per cent. During this time the animal was perfectly flaccid and in a comatose condition. However, immediately after the last blood specimen was collected, muscular twitchings appeared and the dog died in convulsions.

For the purpose of comparing the effect of liver extirpation and severe liver injury on the blood sugar, the appended chart has been prepared. In the fatal case of phosphorus poisoning just described, the blood sugar concentration fell to 0.035 per cent fifteen minutes before death. The lowest blood sugar concentration observed by us in chloroform poisoning was 0.053 per cent. The animal showing this value died twelve hours after this determination was made. In an animal dying of hydrazine sulfate intoxication, the blood sugar gradually fell from 0.108 to 0.042 per cent during a period of twenty-one hours. In the dogs with extirpated livers used by Mann and Magath, death also occurred at these low blood sugar levels. The curves representing our results are therefore similar in character to those obtained by Mann and Magath in dogs after liver extirpation.

These data as well as the recent work on insulin clearly indicate that life processes are interrupted when the concentration of the sugar in the blood falls below a certain level. The symptoms which in all cases are approximately the same may be associated, perhaps, with the condition of "sugar hunger." It is noteworthy that glucose has an antidotal effect in relieving the symptoms associated with marked hypoglycemia whether due to liver extirpation or to the action of insulin. On the other hand, it is stated by Underhill that subcutaneous injection of dextrose into dogs previously treated with non-fatal doses of hydrazine, causes the death of these animals. This statement appears paradoxical in the light of the work just mentioned.

SUMMARY

It has been found that hypoglycemia occurs in severe chloroform poisoning as well as in hydrazine and phosphorus intoxications. This is especially marked in poorly-nourished animals. The initial effect of severe liver damage, produced by these agents, is hyperglycemia. Subsequently this effect disappears and the blood sugar level falls to subnormal values.

Death resulting from acute chloroform and phosphorus poisoning is not entirely due to functional damage of the liver as these substances are general protoplasmic poisons. On the other hand, in chronic conditions of liver injury or in conditions of acute chloroform and phosphorus intoxication in animals with damaged livers, hepatic insufficiency may be the primary cause of death. In such cases we have observed symptoms similar to those described by Mann and Magath in the case of completely hepatectomized dogs.

BIBLIOGRAPHY

(1) Mann and Magath: This Journal, 1921, lv, 285; Arch. Int. Med., 1922, xxx, 77, 171.
(2) Williamson and Mann: This Journal, 1923, lxv, 267.
(3) Hendrix and Sweet: Journ. Biol. Chem., 1923, lv, 161.
(4) Mann: This Journal, 1921, lv, 285.
(5) Frank and Isaac: Arch. exper. Path. u. Pharm., 1911, lxiv, 274.
(6) Underhill: Journ. Biol. Chem., 1911–12, x, 165.

STUDIES IN PERISTALTIC FATIGUE

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During the last few months a series of studies in peristaltic fatigue has been made in order to throw, if possible, some light on the failure of peristalsis subsequent to abdominal operations or obstructions and, in the hope that suggestions for its treatment might be made. The method most generally employed has been that suggested by Trendelenburg (1).

There are two points in technique in which I have slightly altered the Trendelenburg method. The first is that in the connections between the pressure bottle and gut a T-piece was inserted connecting with the pressure recorder, and in all tracings the movement of this pressure recorder is shown immediately above the time line. Secondly, in order to be sure that the pressure bottle was raised on all occasions at a uniform and constant rate. the following device has been employed.

The pressure bottle is suspended by a cord passing over a pulley. On the lower end of the cord is a weight a little more than sufficient to counterbalance the pressure bottle and its connections. In the bight of the cord, below the pulley, two turns are taken around the axle of a clock-work, which when operating acts as a capstain, and imparts a constant movement to cord and bottle. The bottle is lowered again after having been raised, by slipping the turns on the capstain axle by hand.

Sections of guinea pig gut (10 to 11 cm. long) set up in good condition in Ringer's solution respond with peristaltic movements to an increase in pressure of about 15 to 25 mm. of water. In these experiments the rise in pressure has been at rates of 1 mm. per two or per three seconds. In as far as possible the lowest possible effective pressure has been used. Under certain conditions the peristaltic movements thus set up may continue over a very long period of time—half an hour or even more. But more usually they fail after a comparatively short period, sometimes lasting for only two or three minutes.

On raising the pressure bottle the most usual series of events is as follows: there is a distention of the gut produced by the water flowing into it. This usually produces a shortening of the gut with a rise of the

longitudinal lever with, of course, a fall in the piston recorder due to the water entering the gut. Presently a contraction takes place in both circular and longitudinal coats with a rise of the levers, and from the time that the rhythmic contractions begin, a fall in the longitudinal lever, and very commonly in the circular lever, also takes place. This means that the longitudinal coat has lengthened in spite of the fact that the circular coat is becoming more dilated. In some cases both of these changes are of comparatively slight extent. Under these conditions it is of course a little difficult to say as to whether real tonus changes in the two sheets of musculature are occurring. This becomes more possible as the movements become more regular and especially when rise or fall of both levers enables one to deduce a change in tonus of both coats taking place at the same time.

If the pressure is maintained, failure of these peristaltic movements occurs sooner or later. If when the movements cease the pressure be lowered, the gut will after a rest repeat the peristaltic waves on again increasing the pressure, but if the pressure be maintained in some cases the failure seems to be absolute, but more commonly, especially when low pressures are employed, after a longer or shorter interval, from half a minute to three or four minutes, another series of movements occurs and these are frequently of excellent size, but such groups of waves tend to become shorter and shorter and the intervals longer, and irregularities in the movements often appear.

26.4.23. Strip 4. Figure 1. Seven hours after urethane, and first strip taken out. Breathing slow and feeble. Strip taken from lower ileum, gut flabby, dilated, contracted actively when removed, elongated and relaxed when in saline.

5:00 p.m. Pressure raised 12 mm. H_2O. Very marked longitudinal and circular movements.

5:20 p.m. To this time long series of waves, then groups decreasing in length and strength, and increasing intervals with slight rhythmic tone increases in longitudinal muscles accompanying groups.

5:22 p.m. Strychnine 1:1,500,000. Cessation of groups.

5:20 p.m. Pinch. Good local ring.

5:24 p.m. $BaCl_2$. 1:450,000. Good group followed by others.

25.3.23. Strip 1. Figure 2. Guinea pig 1 hour after urethane, mid-jejunum, tonus low, dilated.

11:18 a.m. Pressure 16 mm. H_2O. Good group lasting 2 minutes. Interval 2 minutes and 20 seconds. Rise in both circular and longitudinal tonus. Second group lasts 100 seconds. Interval 3½ minutes. Group lasting 100 seconds.

During the interval the gut was pinched sharply and responded with good local contractions of longitudinal and circular coats, which however did not record. A sharp temporary elevation of pressure of 10 mm. also failed to produce movements.

18.4.23. Strip 4. Six hours after urethane, gut flabby, actively contracted when cold, relaxed when in saline.

Fig. 1

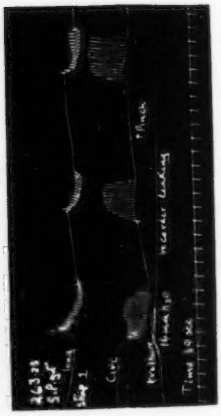

Fig. 2

4:42 p.m. Pressure 15 mm. H_2O. Long series of good circular and longitudinal, lasting till 5:00 p.m.

5:01 p.m. Another group lasting 3 minutes succeeded by other groups, in each group one or two irregular waves, then waves regular in rate increasing in strength to maximum and then abruptly decreasing. Slight rise in longitudinal tonus between groups. Group continued till 5:39: intervals have now increased to 3 minutes. Groups decreased to individual contractions.

5:57 p.m. $BaCl_2$ 1:450,000. Rise in longitudinal lever and good group.

One frequently observes when the pressure is first raised that the initial movements are smaller and more irregular than they subsequently become.

7.5.23. Strip 1. Intestinal strip removed from upper jejunum. Gut active.

9:46 a.m. Pressure raised 21 mm. H_2O. Good peristalsis passing over to group formation in 3 minutes. Between groups small movements with increase in longitudinal tonus.

10:01 a.m. Nicotine 1:600,000. Rise in longitudinal tonus, increased circular movements, then fall of both levers with cessation of movements.

10:01 a.m. $BaCl_2$ 1:150,000. Rise in longitudinal lever, very faint circular movements.

10:15 a.m. $BaCl_2$ 1:112, 500. Rise in longitudinal. No movements.

It may frequently be observed that both the first and last movements of any group are either irregular or of lessser extent than those which occur in the middle of the group. If at the end of one of these groups the gut be pinched there is a local response consisting of a local contraction of the circular and longitudinal muscles which gradually relaxes, e.g., protocols 26.3.23 and 21.5.23 and 18.12.2, figures 2, 4. Such stimulus fails to initiate a group. A sudden rise of pressure also is ineffective in producing movements. Examination of many of these tracings reveals the fact that during the interval there is a slow and often little marked decrease in intestinal volume, figure 1, or shortening of the longitudinal coat; in other words, an increase in tonus in one coat or the other, and frequently in both, figure 2, though movements of the one coat may tend to obscure the movement in the other. It might be suggested, then, that the resumption of the movements is due to a slow improvement in muscular tonus taking place during the interval. I am not at all certain that this conclusion is valid, and I would illustrate my doubt as to whether tonus is the sole factor by a reference to experiment 7.5.23 which is only typical of two or three others which I have seen.

7.5.23. Strip 3. Figure 3. Five hours after urethane, guinea pig in good condition. Gut slightly relaxed, active.

2:30 p.m. Pressure 20 mm. H_2O. Long group lasting 10 minutes. During this period tonus waves in both circular and longitudinal coats. Longitudinal movements increase in height with increasing longitudinal tonus. Circular also but not so markedly.

2:40 p.m. Cessation and fall in both circular and longitudinal levers.

2:43 p.m. Another series. Tonus waves not so marked.

2:54 p.m. Cessation but still small circular rings formed, but no waves of pressure.

2:55 p.m. Pinch. Marked local reaction.

2:56 p.m. $BaCl_2$ 1:300,000. Increased longitudinal tonus and waves irregular, persisting with gradual fall in longitudinal tonus.

3:01 p.m. Nicotine 1:000,000. Marked rise in longitudinal and circular tonus then fall in both, and cessation of movements.

3:05 p.m. Lowered pressure. Rest.

3:07 p.m. Pressure raised to 25 mm.; 8 to 9 irregular movements. Fall of longitudinal lever and cessation of movements.

3:09 p.m. $BaCl_2$ concentration 1:128,000. Slight rise in longitudinal, then long series of irregular movements increasing in strength for ½ hour, then decreasing for ½ hour.

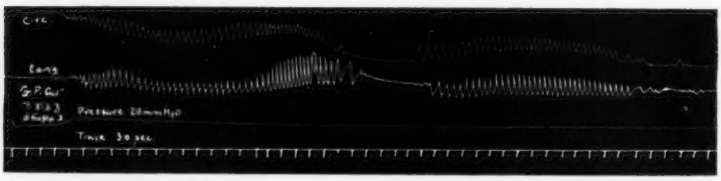

Fig. 3

In this piece of gut tonus waves occurred, and while it is true that the movements tended to improve as the tonus rose, you will note that there was a steady fall of tonus both in longitudinal and circular coats between the two groups shown.

When the failure of movements has occurred they can be very quickly restored as a rule by the administration of pilocarpine in concentrations of 1:2,225,000 to 1:450,000 or barium in concentrations of 1:900,000 to 1:112,000. The resumption of movements after the application of one of these drug-stuffs is often surprisingly long, outlasting the original group many times.

3.5.23. Strip 2. Four hours after urethane, guinea pig in poor condition, respirations feeble, gut empty, collapsed.

2:51 p.m. Pressure 35 mm. Very small longitudinal, small circular. Gradual distention. Ceasing in 3 minutes but followed by 3 smaller groups.

2:55 p.m. $BaCl_2$ 1:224,000. Small group.

2:57 p.m. Same amount. Larger group followed by second much smaller.

2:59 p.m. Pilocarpine 1:225,000. Long series of good movements failing with group formation.

The addition of pilocarpine and of barium in this instance caused very slight, if any, increase in tonus, yet led to a long series of movements.

When movements were failing slow increase in pressure in a few cases caused them to resume.

18.2.23. Strip 1. Strip upper jejunum. One-half hour after urethane. Gut flabby.

10:00 a.m. Pressure 20 mm. H_2O. Movements small in both coats rapidly becoming irregular. Longitudinal lever showing building vibration.

10:16 a.m. Pressure raised 10 mm. Movements more marked and regular.

10:19 a.m. Raised another 10 mm. Little change in movements.

As a rule, however, an increase in pressure tended to cause the movements to cease or did not initiate them if they had failed.

Peristaltic failure did not in all cases occur with group formation, but in some instances the movements ceased when one coat at least was much shorter, the other not obviously more dilated than when the movements were active. This occurred most frequently after the administration of such drugs as pilocarpine or barium.

5.5.23. Strip 3. Lower ileum 3 hours after urethane, gut flabby.

12:18 p.m. Pressure 25 mm. H_2O. Short series, marked relaxation longitudinal and distention circular coats. Pressure lowered.

12:25 p.m. $BaCl_2$ 1:225,000.

12:27 p.m. Pressure 15 mm. Good short series ceasing with longitudinal muscle shorter than during activity.

12:30 p.m. Pressure lowered.

12:31 p.m. Pressure 15 mm. Another group smaller.

As the changes in tonus did not seem to offer an entirely adequate explanation of the resumption of movements after an interval, an attempt was made to ascertain whether these were dependent upon the activity of the cells in the nerve-net. Strychnine applied in concentrations of 1:3,000,000 to 1:1,000,000 in some cases produced, apparently, improvement in the movements as in 21.5.23.

21.5.23. Strip 1. Figure 4. One-half hour after urethane, gut in good condition, upper jejunum.

10:08 a.m. Pressure 30 mm. Group. Pressure lowered. Rate 13 per minute.

10:10 a.m. Pressure 25 mm. Group failing in minutes. Rate 9 per minute.

10:17 a.m. Pinch. Good local response, one wave of peristalsis.

10:18 a.m. Strychnine 1:1,250,000. Slow increase in circular tonus, little waves appear; at 10:22 repeat strychnine, waves increasing in strength but irregular till 10:24.

10:24.30 a.m. Pilocarpine 1:2,250,000. Larger waves. Rate 9 per minute.

10:32 a.m. Removed surrounding Ringer's solution.

10:34 a.m. Removed surrounding Ringer's solution. Lowered pressure.

10:37 a.m. Pressure 25 mm. New series. Rate 17 per minute.

26.4.23. Strip 2. Four hours after urethane gut in good condition. Respirations good; circulation good.

2:46 p.m. Pressure 30 mm. H_2O. Good group of waves lasting 3 minutes. Interval 4 minutes.

2:53 p.m. Group lasting 90 seconds. Interval 5 minutes.

2:58.30 p.m. Group lasting 90 seconds. Interval 5 minutes.

3:03 p.m. Group lasting 60 seconds.

3:05 p.m. Strychnine 1:600,000.

3:07 p.m. (Interval 4 minutes) Group lasting 75 seconds.

3:09.30 p.m. (Interval 80 seconds) Group lesser in height followed by others with irregular intervals not exceeding 50 seconds in any case, for 8 minutes.

Strychnine in other cases had no apparent effects as in 26.4.23, strip 4, figure 1, but rather the reverse.

Nicotine was also tested. The results from the administration of this substance are difficult to interpret. In all cases (see 7.5.23, strip 1) a very marked rise of longitudinal tonus with active movements of both coats of very brief duration was obtained, but was followed by a lengthening of the longitudinal coat, and a relaxation of the circular coat if it had contracted. The movements then ceased. With concentrations of 1:900,000 it was however possible to initiate movements by the subsequent application of barium 1:225,000. If, however, the concen-

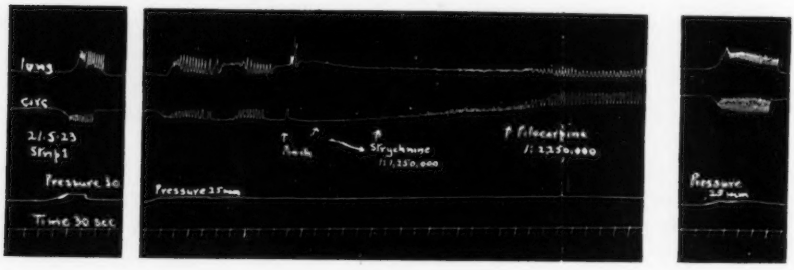

Fig. 4

tration exceeded 1:600,000 no application of barium or pilocarpine would produce movements as in figure 3.

Pilocarpine in concentrations of 1:2,250,000 to 1:250,000 almost invariably initiated a fresh series of waves after failure had occurred or increased the movements when failing. Its action was counteracted by atropine.

18.12.22. Strip 1. Upper jejunum 30 minutes after urethane. Gut in fair tonus.

10:25 a.m. Pressure raised 25 mm. H_2O. Long series lasting till

10:44 a.m. When pilocarpine 1:500,000 was added. Marked shortening of longitudinal coat, little change in gut volume with movements decreasing in both until a slight fall in longitudinal tonus occurred when both movements increased.

10:50 a.m. Pressure raised 10 mm. Shortening of longitudinal muscle with smaller movements.

10:51.30 a.m. Pressure lowered 10 mm. Longitudinal coat shortened greatly. No movements.

10:53 a.m. Pinch. One good local contraction spreading slightly; recorded.

10:55 a.m. $BaCl_2$ 1,225,000. Slight shortening then marked lengthening longitudinal coat and resumption of active movements in both coats.
10:56.30 a.m. Pressure increased 14 mm. Increase in rate of movements.
10:58 a.m. Raised to 40 mm. Failure with distention of longitudinal coat.

Adrenalin in concentrations of 1:1,000,000 to 1:10,000,000 decreased the movements temporarily, and in no case produced movements if absent.

Atropine in concentrations of 1:800,000 to 1:300,000 in most cases tended to diminish the movements of the normal gut in extent and slowly produced their cessation as in 3.5.23.

3.5.23. Strip 1. One-half hour after urethane, upper jejunum gut in good condition.
10:56 a.m. Pressure 25 mm. Good series lasting 2½ minutes when pressure lowered. Rest.
11:03 a.m. Pressure raised 15 mm. Good long series.
11:09 a.m. Atropine 1:225,000. Steady decrease in movement with increasing distention to cessation.

But in other cases this effect was not produced and movements persisted for a long time after atropine.

12.5.23. Strip 1. One-half hour after urethane. Gut in good condition upper jejunum.
10:10 a.m. Pressure 23 mm. Good long series.
10:16 a.m. Atropine 1:300,000. Slight decrease in longitudinal movements.
10:20 a.m. Atropine 1:150,000 or together 1:100,000 gradual decrease in longitudinal movements but circular movements continued unaltered for 16 minutes.

Atropine in some cases decreased or abolished movements produced by barium but in other cases failed to do so.

Cannon (2) in his discussion of the causation of antiperistalsis in the colon and of the so-called peristalsis of the pyloric portion of the stomach ascribes the progress of the waves entirely to a muscular phenomenon. He considers that a contraction of any ring of gut, leading to a displacement of fluid distends the neighboring ring and therefore stimulates it to contraction and thus the ring of activity passes along the gut. His observations in the colon show that at some point a marked tonus ring arises from which regular waves of contraction spread. In the stomach from a similar ring waves arise passing toward the pylorus. When a series of sections through the musculature and nerve-net were made, these waves were conveyed in an almost normal manner. Trendelenburg (1) imitated this experiment by cutting the suspended gut with Pean's clamping forceps which destroyed all but the serous coat. Nevertheless, waves passed over this interval. Trendelenburg concludes that "Der Ablauf der Dunndarm peristalik kann also ebenso wie das

Fortschreiten der Magen peristalik ohne nervöse or musculäre Nervenleitung vor sich gehen." Trendelenburg seems to recognize this as a purely muscular phenomenon, but can hardly explain the origin of the peristaltic waves at the upper end of the gut segment where they always arise in a normal undamaged strip. Nor does Cannon's explanation account for the original ring. Magnus (3) believed that he had proven that pendular movements are impossible in the absence of the nerve net. Gunn and Underhill (4) have, however, shown that rhythmic movements can be carried out by plexus free strips. But it seems hardly probable that such a complex movement as peristalsis in the small intestine is carried out independent of the nerve net. Further, the experiments of both Cannon and Trendelenburg are capable of another explanation. Each area of stomach and gut isolated from its neighbors by transection of muscular coats and net retains its local mechanism. The displacement of fluid from a more proximal ring distending the more distal one sets up an increase in pressure or lengthening of muscle fibers in it which may well serve as the stimulus, setting in action the local mechanism. In the experiments reported above direct proof that Cannon's explanation is not adequate is given. In every case immediately after the conclusion of a series of peristaltic waves a pinch produces a good local contraction. This shows that the muscle is irritable and is not fatigued. In many cases, for example in 18.12.22, strip 1, such a pinch caused such a marked constriction of a local area at the upper end of the gut as to be recorded by both longitudinal and circular recording levers, and the gut could be seen to be completely occluded by the circular muscle and shortened over an area of often $\frac{1}{2}$ to $\frac{3}{4}$ of a centimeter. In 21.5.23, strip 1, figure 4, a single peristaltic wave was produced; yet no series of waves was set up in the area below. Sudden increase in pressure in such an interval between two series of waves is also ineffective as a stimulus. Further, these experiments have shown that a tonus increase does not necessarily, though it usually does, precede a series of waves. The failure must be, not a muscular fatigue, but one taking place in some other structure.

The results of experiments with drugs are frankly more difficult to explain. Strychnine which acts on nerve synapses may be effective in restoring movements, but we have no certain knowledge that strychnine is an effective agent in a nerve-net. Nicotine does seem to be effective but only temporarily. It seems to stimulate the nerve cells but unfortunately such stimulation is always followed by a depression. Pilocarpine is effective but again we have no certainty of its point of attack. It is usually considered to stimulate the vagus endings but whether these lie in the muscle or in the net we do not know. Nor are we entirely sure that the effect is not muscular, as Magnus (3) showed

that a plexus free strip of muscle contracted to pilocarpine. Atropine does not depress vagus endings as to prevent vagus stimulation (5), (6), yet as I have recently shown (7), it decreases the tonus of the gut and this may often be seen in experiments of the type reported in this paper. With fall in muscle tonus, the circular waves especially may persist but cut so little into the lumen as to lead to no displacement of fluid in the peripheral direction. The rings are often rapid and simply seem to run over the surface. Atropine may decrease or abolish the movements in normal gut, possibly removing the effect of a preëxisting choline stimulation (8). It may either cause a decrease of movement after barium or may not do so.

Barium has been looked upon as a pure muscle stimulant. Yet this view is not universally supported. Roth (9) has recently shown that the effect of barium on the pendular movements of the intestine of the rabbit could be antagonized by epinephrin. And very frequently I have found that its effect could be opposed by atropine. That barium fails to act after large doses of nicotine recalls the experiments of Cathcart and Clark (10), who found that it failed to raise blood pressure in the rabbit, at all events to a normal extent after nicotine. Cathcart and Clark conclude "Barium chloride probably does not act directly on the muscle substance of visceral muscle."

I would explain my results as due to a depression of the cells in the nerve net by the nicotine, and this would possibly explain those of Cathcart and Clark. When nicotine has not been used, even if we may assume that barium acts on muscle substance, its effect may simply be due to its putting the muscle in such a condition that the nerve-net may more easily produce its effect upon the muscle. Pilocarpine by stimulating vagus ending may possibly produce a similar effect.

Trendelenburg (1) asserts that the sole factor in determining the rate at which peristaltic movements sweep over the gut is the length of the strip. That this is not the case is abundantly shown by 18.12.22, strip 1, or by 21.5.23, strip 1, figure 4, which is typical of other cases. In this strip a pressure of 30 mm. produced waves at a rate of 13 per minute, a pressure of 25 mm. at a rate of 9 per minute, while the same pressure at a later period after strychnine and pilocarpine produced waves at a rate of 17 per minute. Such results may be due to a differing response of the net to different pressures, to different synaptic resistances or differing muscular states.

CONCLUSIONS

1. Peristaltic failure is not due to a failure of the activity of the muscular coats alone.

2. Evidence is presented which suggests that it is due in part at least to fatigue of the nerve-net.

3. Activity may be restored in most cases by barium chloride or pilocarpine, and under certain conditions, possibly by strychnine.

BIBLIOGRAPHY

(1) TRENDELENBURG: Arch. f. exper. Path. u. Pharm., 1917, lxxxi, 55.
(2) CANNON: This Journal, 1911, xxix, 238, 250.
(3) MAGNUS: Arch. f. d. gesammt. Physiol., 1904, cii, 349.
(4) GUNN AND UNDERHILL: Quart. Journ. Physiol., 1914, viii, 275.
(5) BAYLISS AND STARLING: Journ. Physiol., 1899, xx, 99.
(6) CUSHNY: Journ. Physiol., 1910, xli, 233.
(7) HENDERSON: Arch. Intern. de. Pharm., 1922, xxvii, 205.
(8) LE HEUX: Arch. f. d. gesammt. Physiol., 1920, clxxix, 177.
(9) ROTH: Proc. Soc. Exper. Biol. and Med., 1920, xx, 43; Arch. intern. de Pharm., 1923, xxvii, 333.
(10) CATHCART AND CLARK: Journ. Physiol., 1915, l, 119.

REFLEX CONTRACTIONS OF AN ALL-OR-NONE CHARACTER IN THE SPINAL CAT

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It has become almost a commonplace in the introduction to a paper on all-or-none phenomena to say that they are widely accepted for muscle and nerve. Pratt and Eisenberger's (1), recent admirable work on the stimulation of individual muscle fibers must be convincing to the most skeptical and the cumulative evidence is becoming almost as strong that the law applies equally to the neurone. Discussion, summaries and literature are to be found in various recent papers (cf. Martin (2) and Adrian and Forbes (3)). A paper of Mines (4) has a particular bearing on results which will be described later in this article. One of his records is reproduced in figure 1. On applying single shocks, gradually increased in strength, to the uncurarized sartorius preparation from the frog he found the muscle responding in steps, each step of an all-or-none character. In the light of previous researches he interpreted each step to mean the stimulation of a fresh neurone and the contraction of all the muscle fibers innervated by it. Any gradation in the extent of contraction is brought about only by increasing or decreasing the number of neuro-muscular units, not by gradation in the extent of contraction of the muscle fibers. We have obtained essentially similar records for mammalian nerve-muscle preparations.

The question has arisen as to whether in the case of reflex response to single shocks the central nervous system is able to overcome the all-or-none limitations of the muscle cell and nerve fiber and produce finer gradations of response than is possible for these structures when they form a nerve-muscle preparation. Is there "something at the synapse which passes into a sustained state of activity uninterrupted by refractory phase, and capable perhaps of gradation"(5)? Suppose, for example, the arc is able to transform the single afferent impulse aroused by a single shock into a shower of impulses along the motor neurone. If such showers varied in the number of constituent impulses and in the intervals between them, they might conceivably give almost any degree of muscle contraction height between that of the

first twitch and a final complete tetanus. Examples of such gradation for a whole muscle can be found in any textbook of physiology in the figures illustrating the development of tetanus. Sherrington is of the opinion that such showers do arise in the arc. "Even when initiated by so brief a stimulus as a single induction shock the reflex action seems to involve some central process whose result is repetitive discharge of impulses" (6). He does not assert that a reflex discharge is always repetitive. In fact he accepts Snyder's (7) and Jolly's (8) evidence that the knee-jerk, for example, is a single twitch, caused by a single motor impulse. It follows that gradation in the knee-jerk must be dependent on the number of motor neurones receiving this single stimulus, not on the varying extent of contraction of muscle fibers.

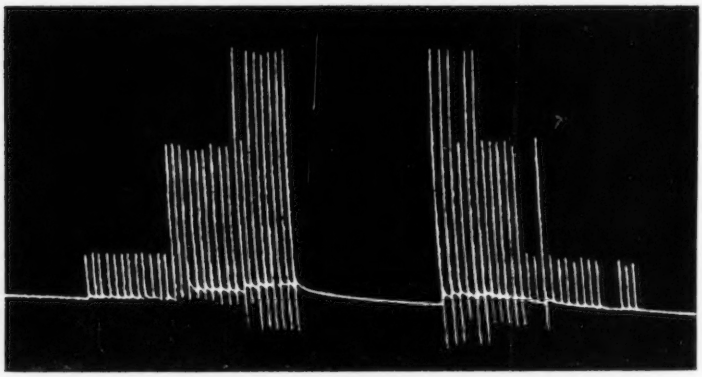


Fig. 1. Uncurarized sartorius from the frog. Step-like rise and fall in contraction height as stimulation is gradually increased and decreased (4).

Our object in the present research was to study the gradations of response to gradually increasing shocks, starting below the threshold, in a reflex employing a very small muscle and one receiving few motor neurones. We proposed to compare this with similar responses from the same muscle when stimulated through its motor nerve, i.e., we were to compare reflex with nerve-muscle responses. If the records should turn out to be essentially alike it would be at least presumptive evidence that the nerve impulses aroused reflexly were of the same character as those aroused by direct stimulation of the motor nerve. If on the other hand we should discover that reflex response, instead of showing a step-like character, showed a steady and gradual rise in contraction height as the sensory nerve was stimulated more and more strongly, we should be forced to conclude that the central nervous

system was in some way modifying the single impulses received from the sensory nerve, possibly by sending out repetitive discharges as Sherrington has suggested, and that the reflex arc, therefore, was not limited by the all-or-none behavior of its constituent elements. We proposed to study only responses at or near the threshold so that there should be no possibility of a single shock to the sensory nerve causing more than a single sensory nerve impulse. Forbes and Gregg (9) have shown that with strong stimuli this may happen.

METHOD. The muscle employed was the tenuissimus found regularly in the carnivora but not in all mammals (10). A research of Graham Brown (11) along lines similar to our own brought it to our attention. As he has pointed out, this muscle is admirably adapted for the study of minute reflex movement. It is a long, slender, straight fibered muscle, underlying the biceps, originating on the caudal vertebrae and inserted halfway down the lower leg as a continuation of the most distal portion of the biceps insertion. It is of about the size and proportions of the lead in a common lead pencil, except that it is a flat band. We have counted the fibers in a cross-section and find there are approximately a thousand. An idea of its minute size may be gained from the fact that the tibialis anticus muscle in the cat, a relatively small one, contains, we estimate, sixteen thousand fibers in a cross-section at its largest part. Tenuissimus is innervated 2 or 3 cm. from its origin by a tiny branch from the sciatic. This divides into two branches about 5 mm. from the muscle. We have not determined the number of fibers in the nerve but counts made by Brown (12) on one of the two branches indicate a total for the entire nerve of from 50 to 100 fibers, varying in different animals. If tenuissimus resembles other muscles in the cat, one-half to two-thirds of these are motor (13). In a nerve containing 75 fibers, therefore, we may suppose 50 to be motor, each motor neurone innervating 20 muscle fibers. This agrees with Kölliker's (14) estimate for the sterno-radialis of the frog. It was not necessary in this research to know the figure accurately since it was not proposed to utilize all the fibers but only a few near the threshold. Tenuissimus is one of the large group of muscles taking part in the flexion reflex. It responds to single shocks and often at about the same threshold found by one of us for the flexion reflex as a whole, and for the tibialis anticus (15).

The animal is made low spinal by the method of pithing brain and thoracic cord described by one of us in a previous paper (16). All the muscles of both hind legs are paralyzed by nerve section, with the exception of tenuissimus and one or two other small muscles whose nerves were difficult to get at. Tenuissimus is prepared for graphic registration as follows: A cut several centimeters long is made on the

side of the leg, over the biceps. Biceps is cut along its insertion on the lower leg, reflected, its blood supply tied off, with care not to interfere with the circulation of tenuissimus, and it is excised with the exception of a small portion near its upper end. Tenuissimus is thus exposed for several centimeters. Its nerve is located and the muscle itself carefully dissected free for 2 or 3 cm. below the entrance of the nerve. It is then ligatured and cut 1.5 to 2 cm. distal to the nerve. This sacrifices an artery entering the muscle below the ligature but other vessels enter higher up near the nerve.

The contraction of the muscle is recorded optically. One arm of a Harvard Apparatus Co's. heart lever is cut off and the other end, about 5 cm. long, hangs vertically down into the wound and is attached to the muscle, which lies in a horizontal position. The lever carries on its axis a bit of galvanometer mirror 5 mm. in diameter. The edges of the wound are drawn up by clips and weights so that the wound forms a trough or cup. This is kept filled with Ringer's solution. A piece of watch spring straightened out is clamped at one end to an upright and its other end caused to press lightly against the lever with sufficient tension just to hold the muscle in a horizontal position as it lies free in the Ringer's solution. The tension on the muscle, developed by the spring, is 0.2 to 0.5 gram.

The optical registration is an improvised modification of that commonly employed for cardiographic work. An arc lamp having an adjustable horizontal slit in front of it is focused on the mirror of the muscle lever. The light reaches the camera as a horizontal line 1 to 2 mm. broad. The camera is merely an ordinary kymograph with its spindle projecting through a platform. Over the drum and resting on the platform is a cubical tin box with a vertical slit on one side as long as the drum. In front of the slit is a cylindrical lens focusing the light on the drum surface. "Photowrit" paper (Defender Photo Supply Co.) is clipped around the drum. An extra large fan reduces the speed of the drum to one revolution in 25 minutes. By the use of appropriate screens to exclude extraneous light from the camera the records may be secured without darkening the room. The slow movement of the drum results in an over-exposure of the base line and some fogging in its vicinity, but the first contraction height is usually great enough to carry the record well out of the darkened region on the paper. The undulating base line seen in some records is due to the movements of artificial respiration being communicated to the muscle. This sometimes causes slight irregularities in contraction height. It is probable that the essential features of the contractions could be recorded by delicate apparatus on a smoked drum, but having adopted the optical method it has been adhered to. Magnification has usually been about 10 ×.

Early in the course of the research a difficulty was experienced which until overcome threatened to defeat the purpose of the investigation. Almost without exception at about the time preparations were complete for recording, the muscle began to show spontaneous twitchings. These were at first minute, involving only the first increment of contraction but soon became larger and more frequent until the muscle passed into an irregular clonus or almost a tetanus. The cause of this twitching was finally determined to be irritation at the edges of the wound in the skin, i.e., the movements were really flexion reflexes in response to "pain." After various unsuccessful devices to allay this irritation had been employed the solution was finally found in the use of "anesthesin" (ethyl amino benzoate) or "orthoform" (methyl metaaminoparaoxybenzoate). These difficultly soluble compounds when applied to mucous membrane or a raw surface act as local anesthetics. They are absorbed very slowly and are reported to be practically non-irritant and non-toxic (17), (18). The drug was prevented from reaching nerves other than those at the skin wound by covering other surfaces with a layer of cotton before filling the wound with Ringer's solution or applying the drug. The recording portion of tenuissimus was of course left free. The anesthesin was applied by dusting with a cotton swab along the skin edges and underneath the skin as far as it had been loosened. Immediate success followed its use. Twitchings gave no further trouble, while reflex movement on stimulating the appropriate sensory nerve could be obtained with as low a threshold as ever. Under these circumstances the reflex may remain for several hours with little change in threshold or amplitude of contraction.

A form of liquid electrode described in a previous paper (19) was used for stimulation, the indifferent electrode being the tracheal cannula. The liquid electrode was made the physical anode, since its form is such that when applied as described it must produce within the nerve a physiological cathode which is the seat of stimulation. Break induction shocks have been used and the makes have not been short-circuited, but they have always been too weak to cause stimulation. Makes occurred from 1 to 2 seconds before breaks. Breaks were given at varying intervals in different experiments but always at least 3 seconds apart. The primary circuit has been made and broken by means of an ingenious key devised by Pratt (20). A small rubber tube filled with mercury is alternately compressed and released at one point, the mercury column being thereby broken and then allowed to flow together again. In our form of the key the compressor was an extension fastened to the bar of a telegraph sounder, the current through the sounder being controlled by an automatic circuit breaker. This simple key is believed to deliver shocks of great uniformity.

It was desirable that the increase in the strength of shock to the nerve should be very slow, gradual and steady. This was brought about by mounting a long threaded rod or screw alongside the secondary. An adjustable pinch clamp, also threaded, attached to the secondary permitted the secondary to engage the threads of the long screw at any point desired. The long screw bore a grooved pulley at its upper end. This was turned by clock work, the speed of which could be varied. Usually an advance of the secondary of from 2 to 3 cm. covered all the steps it was desired to record. Thus in *a*, figure 3, the secondary, starting 6.7 cm. away from the primary, was advanced 2.3 cm. toward it, this increase in shock causing the appearance of four steps in contraction height. Unless otherwise stated the primary current has been 0.1 amp. Its source is a storage battery. For the convenience of those familiar with the Z-units of Martin (21) the strength of break shock is given in these units as well as indicated by the position of the secondary coil. This coil is a large one of the du Bois-Reymond type with secondary 15 cm. long. The secondary begins to over-ride the primary at the 15 cm. reading. This means that for the experiments cited, the secondary was from 2 to 10 cm. away from the primary.

For reflex contractions the electrode has been applied to the posterior tibial nerve just back of the tendo achillis. In one or two cases the internal popliteal above the knee has been employed. For nerve-muscle contraction the electrode has been applied to that half of the sciatic which is later to become the external popliteal. It is from this half that the nerve to tenuissimus springs. The sciatic trunk is carefully split into two portions. The appropriate portion is ligatured and cut as high up as possible and drawn into an electrode of the same type as that used for reflex stimulation.

Results. When the motor nerve to tenuissimus is thus stimulated by gradually increasing shocks, starting below the threshold, the resulting record of nerve-muscle contractions (see fig. 2) is in all essential features like that obtained by Mines from the sartorius preparation from the frog. (All figures except figure 1 are to be read from right to left.) The increase in contraction height as the shock increases in strength is not gradual but by a series of step-like increments, each step of an all-or-none character. Near the threshold of any given step there are a good many irregularities, steps dropping out and reappearing, but always, as Mines observed, falling into line with one or other of the steps. The balance is so delicate that influences beyond experimental control—by our technique at least—at one moment permit excitation of a given neurone, at another moment prevent it.

Often four, five or even more steps can be identified with the greatest distinctness. Records have not been made of more than that number, both because they become less regular, and because of the amplitude, which carries them off the drum. The purpose of the research was satisfied if even two or three increments were registered regularly and clearly. These definite steps or increments are taken to indicate, just as in Mines' experiment, that one neurone after another comes into activity, as the current, slowly increasing in strength, reaches the neurone's threshold value.

If now tenuissimus is made to contract reflexly, from stimulation of the posterior tibial nerve by gradually increasing shocks, the record strikingly resembles the nerve-muscle record (fig. 3, *a*). There are the same step-like increments of contraction of an all-or-none character, and, what is of more significance perhaps, the increments are of the same order of magnitude as the nerve-muscle increments.

Four or five steps are often clearly distinguishable, just as in nerve-muscle records. Obviously such comparisons between records are more valuable if made from the same muscle in the same animal. In several cases we have been able to do this, first securing our reflex record and then shifting the electrode to the motor nerve. The two records of figure 3 were obtained in this way. The reflex here shows four steps in reaching the height attained by the nerve-muscle in two but the correspondence in steps is often much closer than this, as for example, in figure 4, where both records are again from the same muscle. Here almost identical steps in contraction height may be distinguished in the two records. Both are unusually irregular in the intermixture of high and low steps but the heights of the various steps are clear in spite of this. The movements of artificial respiration are responsible for some of the smaller irregularities in the nerve-muscle record. There is no indication that the reflex produced any better grading of response than the nerve-muscle preparation. Both show roughly the same number of steps in reaching a given contraction height.

Consideration of the conditions under which stimulation is effected will show that any exact correspondence between two such records is not to be expected. It is a matter of pure chance with a nerve-muscle preparation that our increasing shocks reach first the same neurone which was first brought into action by the reflex arc. That neurone may be near the surface of the motor nerve and receive the first effective stimulus or it may be deeply imbedded and protected from the current. It is not at all likely furthermore that each motor neurone innervates the same number of muscle fibers. It is quite conceivable that one increment may represent the contraction of 40 muscle fibers, another 10 and a third 30. The wide variations in increment height often seen

Fig. 2

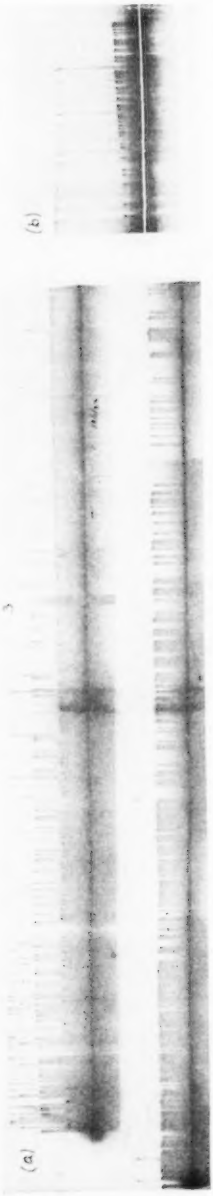


Fig. 3

Fig. 5

Fig. 2. Tenuissimus nerve-muscle preparation in the spinal cat. Step-like increments in contraction height as stimulation is gradually increased. Read from right to left.

Secondary advanced from 22 cm. to 20 cm. on the inductorium scale (23.3 to 32.9 Z-units). Electrode on small portion of sciatic. Primary current 0.1 amp. The undulations in the base line were caused by the movements of artificial respiration. This was partially responsible for slight irregularities in contraction height. Original magnification ca 10×. Reduced two-thirds. Duration of record 25 mm. Stimulation 15 per minute.

Fig. 3. *a*, Tenuissimus reflex preparation. Spinal cat. Electrode on posterior tibial nerve. Step-like increments in contraction height of an all-or-none character on gradually increasing stimulus. Read from right to left. Note similarity to nerve-muscle record in figure 1. Record begins on lower line, continued on upper. Secondary advanced from 21.7 cm. to 19.4 cm. on scale. (24 to 36 Z-units.) Duration of record 50 minutes. Stimulation 20 per minute.

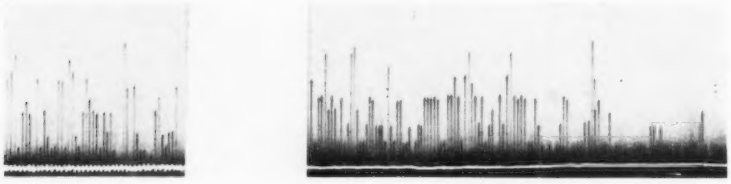
b, Nerve-muscle record from the same muscle as in *a*, 1½ hours later. Electrode on very small portion of sciatic. First and second increments roughly of same order of magnitude as in *a*. Secondary advanced from 17.8 cm. to 17.4 cm. (52 to 53 Z-units). Stimulation 20 per minute.

Original magnification ca 10×. Reduced two thirds.

Fig. 5. Tenussimus reflex preparation in the spinal cat. Read from right to left. This reflex record shows an evenness and regularity in its first increment of contraction which had not been exceeded by any nerve-muscle record secured. Electrode on posterior tibial nerve. Secondary advanced from 26.6 cm. to 23.2 cm. on the scale (12.4 to 19.4 Z-units). Duration of record 22 minutes. Stimulation 20 per minute. Base line retouched in white. Original magnification 10×. Reduced two-thirds

in the contractions of any given nerve-muscle preparation suggest this. Hence no exact correspondence in steps of reflex and nerve-muscle records is to be expected. The reflex increments are sometimes higher, sometimes lower, than nerve-muscle increments, but always roughly of the same order of magnitude. This is the significant thing. While not conclusive, it is strongly presumptive evidence that the impulses aroused reflexively in the motor nerve do not differ from those aroused by electrical stimulation.

These records also throw some light on the question of the variability of reflex threshold as compared with that of the nerve-muscle. It is the accepted belief that the threshold of the reflex is more variable than that of the nerve-muscle. The variations have been measured and plotted by one of us and reported in a previous paper (22). Using the tibialis anticus for reflex contractions and an extensor muscle of forelimb for the nerve-muscle preparation, the thresholds were followed



Nerve-muscle Reflex

Fig. 4. Reflex and nerve-muscle records from the same tenuissimus muscle. Note close correspondence between increments of contraction in the two records. Base line retouched in white. Undulations in base line due to artificial respiration. Some irregularities in contraction height are referable to this.

over periods of half an hour and the reflex threshold found to vary more than that of the nerve-muscle preparation. But the method of recording was coarse compared with the present one. Variability in threshold should be shown on the present records by the appearance and disappearance of any given increment, particularly near its threshold value. If its threshold varies it should drop out from time to time and reappear again. But whereas this does occur, it occurs in both reflex and nerve-muscle records and no more often in one than in the other. We have never secured a more regular and unvarying nerve-muscle record than the reflex record shown in figure 5—particularly as regards the first increment. May it not be that the variations in threshold only appear noticeably when we are dealing with a reflex mechanism more complicated than the simple arcs represented by these first few increments? Some observations on the effect of strychnine on reflex threshold reported by one of us (23) bear on this point. It was found that whereas strychnine invariably lowered the threshold for the

crossed-extension reflex, in many cases no change for the threshold of flexion in the tibialis anticus muscle could be demonstrated. This simple arc seemed to be often of the lowest threshold of which it was capable, at least so far as strychnine could affect it.

That the reflex arc is in general more susceptible than the nerve trunk to certain changes in its environment is well recognized. One need only mention the effect of anesthetics, or lack of oxygen. But it may well be that in experiments such as are described in this paper the changes in environment are so slight as to have no more effect on the arc than they do on the nerve-muscle preparation. Hence the regularity of the first increment in such a record as figure 5 and the lack of evidence of variation in threshold.

We are impressed with the importance of the first increment or two of contraction as a means of studying reflex physiology further. "Some technique is urgently needed by which the (reflex) activity of a single nerve-fibril can be observed and followed" (24). We believe the methods described in this paper approximately supply this technique and meet the need. We cannot assert that we have but one sensory, one association and one motor fibril concerned in our first reflex step of contraction, but it does appear to us probable that we have a reflex reduced to its fewest possible components. Comparison with the nerve-muscle record strongly indicates that the final reflex link is as simple as the final nerve-muscle link, almost certainly one motor neurone with its attached muscle fibers. It seems to us that a further study of the physiology of this ultimate reflex unit, or reflex at its lowest terms, may serve to differentiate between those characteristics inseparably connected with conduction through the simplest reflex arc and those only found when the reflex mechanism becomes more complex. For example, irreversibility of conduction is always a characteristic of the arc; variation in reflex threshold, on the other hand, appears from our results not to be present so constantly or to such a marked degree as has been supposed.

SUMMARY AND CONCLUSIONS

A mammalian preparation—the tenuissimus muscle in the spinal cat—when stimulated by gradually increasing shocks delivered to its motor nerve, responds near the threshold by step-like increments of contraction height which have an all-or-none character, thus resembling similar preparations in the frog.

When the tenuissimus is caused to contract reflexly by gradually increasing shocks to the appropriate sensory nerve the muscle responds by similar all-or-none step-like increments which are of the same order of magnitude as those exhibited by the nerve-muscle preparation.

This is regarded as presumptive evidence that the motor nerve when stimulated near the threshold by the reflex mechanism of the cord is stimulated neurone by neurone just as it is when stimulated by shocks applied directly to it.

There is no conclusive evidence of a finer gradation of reflex response in the first few steps in contraction height than is produced by the nerve-muscle preparation.

Such finer gradation as a result of summation would be likely to appear if the arc produced repetitive discharge. To this extent, therefore, the results are against the view that the arc invariably produces repetitive discharge.

A continuous record of the reflex contractions involved in the first increment or two, although showing irregularities caused by increments dropping out from time to time, is often as regular as a similar nerve-muscle record. This is against the view that the reflex threshold for the very simplest arcs is more variable than that of nerve-muscle preparation.

The resemblance between the reflex steps and the nerve-muscle steps suggests that the reflex as represented by the first step is a simple affair, possibly involving but one sensory, one association and one motor neurone. Whether or not this be true it may be regarded in any event as a reflex reduced to lowest terms. It is suggested that a study of such a reflex may serve to give further information as to the qualities inseparably connected with the simplest arc as distinguished on the one hand from the nerve-muscle preparation, and on the other from reflex arcs of greater complexity.

BIBLIOGRAPHY

(1) Pratt and Eisenberger: This Journal, 1919, xlix, 1.
(2) Martin: This Journal, 1922, lix, 400.
(3) Adrian and Forbes: Journ. Physiol., 1922, lvi, 301.
(4) Mines: Journ. Physiol., 1913, xlvi, 1.
(5) Forbes: Physiol. Reviews, 1922, ii, 361.
(6) Sherrington: Proc. Roy. Soc. B., 1921, xcii, 245.
(7) Snyder: This Journal, 1914, xxxv, 340.
(8) Jolly: Quart. Journ. Exper. Physiol., 1911, iv, 67.
(9) Forbes and Gregg: This Journal, 1915, xxxix, 172.
(10) Klaatsch: Morphol. Jahrb., 1901, xxix, 217.
(11) Brown: Proc. Roy. Soc. B., 1914, lxxxvii, 132.
(12) Brown: Loc. cit.
(13) Sherrington: Journ. Physiol., 1894, xvii, 211.
(14) Cited in Böhm, Davidoff, Huber: Text-book of Histology, 2nd ed., 1905, 162
(15) Porter: This Journal, 1912, xxxi, 141, 1917, xliii, 497.
(16) Porter: This Journal, 1912, xxxi, 141.
(17) New and Non-official Remedies, Amer. Med. Assoc., Chicago, 1922, 39.

(18) SEIFERT: Die Nebenwirkungen der modernen Arzneimittel. Wurzburg, 1915, 44 and 79.
(19) PORTER: This Journal, 1917, xliii, 497.
(20) Personal communication.
(21) MARTIN: The measurement of induction shocks. New York, 1912.
(22) PORTER: Loc. cit.
(23) PORTER: This Journal, 1915, xxxvi, 171.
(24) HOLT: Psychol. Bull., 1914, xi, 145.

FURTHER OBSERVATIONS ON THE ANTIGENIC EFFECTS OF SEMEN

Mechanism of Sterilization of Female Rat from Injections of Spermatozoa

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In a previous article (1) I presented results indicating that female rats may be rendered sterile for considerable time by the subcutaneous injection of rat spermatozoa or extract of rat testis; furthermore that such sterility may be due to the presence in the vaginal and uterine secretions of the injected rats of substances that immobilize and agglutinate the spermatozoa. The duration of sterility induced in this way seemed to be dependent to some degree on the amount of the material injected, but the oestrous cycle did not seem to be modified by such injections. In adult male rats injections of rat spermatozoa or extract of testis appear to result in destruction of spermatozoa and some cases in atrophy of the testes. The bearing of these results on the work of Guyer (2) and Dittler (3) was pointed out, and the earlier literature on "spermatoxins" and sperm antibodies was reviewed. Recently Mayer (4) and Vogt (5) report clinical observations that tend to support my results. Thus Mayer states that in women abortion of young ova may follow frequent coitus after long abstinence; that ovarian follicles may burst frequently from sexual intemperance, hindering the formation of the corpus luteum, which is essential for the embedding of the ovum. Vogt assumes that sperm overloading may cause sterility which subsides after prolonged abstinence; the fact that conception frequently results after the first coitus may be explained on the score that there is no immunity to spermatozoa; and further that the stimulus of sperm proteins may increase the capacity of hypoplastic genitals and promote the cure of chlorosis. Tsukahara (6), working with horse and beef materials, concludes that male sex cells contain peculiar proteins because he found that injections in the same species of testis extracts result in the formation of specific antibodies (precipitins, complement fixing bodies, Abderhalden bodies), and he assumes that such antibodies may develop after conception.

Further experiments. Further breeding experiments have been made in the same way as described previously (1). The vaginal secretion of immunized rats was studied as before for its action on spermatozoa at the same time as the blood serum of the same animals was tested also for specific precipitins by the layer or contact method, as well as for direct action on the spermatozoa. For the latter purpose a drop or two of a suspension of active spermatozoa was placed in a hollow slide and a drop of serum or vaginal secretion added. The pit was sealed by a cover slip and the slide left at room temperature, and examined from time to time. The precipitin test was carried out as follows: A small amount of perfectly clear seminal fluid diluted in salt solution was

TABLE I

Breeding experiments after subcutaneous injection of spermatozoa in female rats

AMOUNT OF SUSPENSION INJECTED	TOTAL NUMBER OF RATS	NUMBER OF RATS BECOMING PREGNANT	TIME IN WEEKS BETWEEN MATING AND CASTING OF FIRST LITTER			AVERAGE NUMBER OF YOUNG TO EACH LITTER
			Minimum	Maximum	Average	
0.5 cc.: 40,000 spermatozoa	14	14	4	30	12.5	4.6
1.0 cc.: 80,000 spermatozoa	62	57*	4	30	11.8	4.6
3.0 cc.: 120,000 spermatozoa	5	2†	9	30	14.8	3.5
Control: Injection of salivary gland ext.	4	4	3	5	4.0	7.7
	4	4	3	5	4.0	7.7
Control: Normal rats not injected	15	15	3	6	4.0	6.9

* Five rats had not cast litters at the end of the period of observation, 30 weeks.

† Two rats died, non-pregnant; one 5 weeks after injection, and the other 15 weeks. One rat had not yet become pregnant at the end of observation, 30 weeks.

placed in small glass tubes and a little serum introduced at the bottom of each tube in such a way as to secure a precise line of contact between the two fluids. The tubes were left at room temperature for one hour when they were carefully examined for the presence of a greyish white precipitate at the plane of contact between the fluids.

Table 1 gives the results of 100 breeding experiments after the injection of rat sperm. The results indicate that the injection may render the female rat sterile for from 2 to 27 weeks. In few cases only was the injection of sperm suspension without any effect, the rats in these cases casting their litters after the usual gestation period of 4 weeks. The table also shows that while the litter of the normal rat averaged about 7 young, the litter of the injected rats averages about 4.

TABLE 2

Action of vaginal secretion and serum of immunized rats on rat spermatozoa

AMOUNT OF SUSPENSION OF SPERM INJECTED	VAGINAL SECRETION		BLOOD SERUM		PRECIPITIN TITER
	Immobilization	Agglutination	Immobilization	Agglutination	
3 cc.: 120,000 spermatozoa	+++	++	++	++	320
6 cc.: 240,000 spermatozoa	+++	++	++	++	640
9 cc.: 360,000 spermatozoa	+++	++	++	++	640
Control: Normal pregnant rat	+	0	+	+	32
Control: Normal virgin rat	0	0	0	0	0

+++ Reaction in less than 10 minutes.
++ Reaction in less than 30 minutes, but more than 10.
+ Reaction in less than 2 hours, but more than 30 minutes.
0 No effect in 2 hours.

TABLE 3

Development of antibodies after vaginal injection of 40,000 spermatozoa

RATS	TIME AFTER INJECTION		
	7 Days	14 Days	24 Days
1	+	++	++
2	+	+	+
3	0	+	+
4	++	++	++
5	+	+	++
6	+	++	++
Normal rat	0	0	0

++ Immotile in less than 30 minutes.
+ Immotile in less than 2 hours, but more than 30 minutes.
0 Not immotile in 2 hours.

TABLE 4

Development of antibodies in blood for spermatozoa after vasoligation

RATS	7 DAYS		14 DAYS		21 DAYS	
	Immotility	Precipitin Titer	Immotility	Precipitin Titer	Immotility	Precipitin Titer
	min.		*min.*		*min.*	
Vasoligation	3	40	5	40	4	80
Vasoligation	8	40	2	320	2	320

Table 2 gives the results of tests of the vaginal secretion and of the serum of the injected rats with reference to their antisperm action. Both the secretion and the serum immobilized and agglutinated rat spermatozoa and gave a marked increase in precipitin content for rat seminal fluid.

Table 2 also shows that in the normal pregnant rat the vaginal secretion and the blood serum may contain antibodies for rat spermatozoa and precipitins for rat seminal fluid.

Six rats were injected into vagina with 0.5 cc. of active sperm suspension, containing about 40,000 spermatozoa, and the opening sealed with collodion. The results of the tests of blood serum, given in table 3, indicate that spermatozoa introduced into the vagina may exercise antigenic effects.

Table 4 gives the results of tests with the serum of male rats after vaso ligation, that indicate auto-absorption of spermatozoa.

SUMMARY AND CONCLUSIONS

Female rats may be sterilized for a period of 2 to 27 weeks or longer by subcutaneous injections of rat spermatozoa. Such rats when becoming fertile cast smaller litters than normal rats.

The sterility seems to be due to the presence of antibodies for spermatozoa in the vaginal secretions and blood serum. In normal pregnant rats the blood serum and the vaginal secretion may show the presence of such antibodies in small amounts.

Antigenic effects may be exercised by spermatozoa introduced into the vagina and in the male rat vasoligation may be followed by the development of antibodies for semen in the blood.

Immunization with spermatozoa does not affect the sexual cycle of the female rat.

BIBLIOGRAPHY

(1) McCartney: This Journal, 1923, lxiii, 207.
(2) Guyer: Amer. Naturalist, 1922, lv, 637.
(3) Dittler: Münch. med. Wochenschr., 1920, lxvii, 1495.
(4) Mayer: Klin. Wochenschr., 1922, i, 1142.
(5) Vogt: Klin. Wochenschr., 1922, i, 1144.
(6) Tsukahara: Zeitschr. f. Immunitätsf. u. exper. Therap., 1922, xxxiv, 444.

ADRENAL DISCHARGE IN THE DOG

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Cannon (1) and his co-workers, using the denervated heart of the cat as an indicator, Hartman (2) and Hartman, McCordock and Loder (3), using the denervated pupil of the cat, have shown that during pain, asphyxia and excitement an adrenin-like effect is produced which is not obtained when the lumbo-adrenal veins are ligated, when the adrenal glands are denervated or after these glands have been extirpated. Stewart and Rogoff (4), on the other hand, have not confirmed these results.

The use of the denervated heart as an indicator of the reflex discharge of adrenin was suggested by the experiments of Gasser and Meek (5) and of Levy (6) on the dog. These investigators found that there was a marked increase in the rate of beat of the denervated heart of the dog during asphyxia which was not present when the veins from the adrenals were ligated.

In view of the conflicting evidence, some of Cannon's experiments on the cat were repeated on the dog. The technique described by Gasser and Meek (5) was used for the removal of the stellate ganglia. The usual procedure in the succeeding steps of the experiment follow. From ten to fourteen days after the removal of the ganglia the dog was deprived of food for twenty-four to forty-eight hours. There were five exceptions to this rule. Under ether anesthesia the neck was entered through an incision in the scar of the previous operation and a 2 cm. section of the vago-sympathetic trunk on each side was removed at the level of the inferior cervical ganglion. The sciatic nerve was exposed and electrodes from an inductorium were attached to it. A cannula for recording blood pressure on a mercury manometer was placed in the femoral artery of the opposite leg. At this time the heart rate during sciatic stimulation with the tetanizing current and during asphyxia was recorded.

The abdomen was now opened by an incision through the linea alba and with the legs flexed slightly to relax the abdominal wall the lumbo-adrenal veins were located. This is best done by first locating the

hepato-renal ligament. This fold of the peritoneum must be cut nearly to its reflexion onto the posterior abdominal wall. If the liver is now pushed gently cephalad, with the intestines and mesentery pushed to the left, the junction of the right lumbo-adrenal vein with the vena cava is seen. The left lumbo-adrenal vein may then be seen through the peritoneum as it enters the vena cava a few millimeters caudad. Long ligatures of coarse cotton thread were placed about these veins and the ends of each ligature were drawn through a long glass tube (a blunt cannula) with the narrow opening next to the vein. The abdomen was closed with the tubes containing the ligatures protruding through the incision. In five of the dogs this procedure was varied by placing the ligature around the vena cava just cephalad to the entrance of the right lumbo-adrenal vein.

Following the placing of the ligatures another record was made during sciatic stimulation or asphyxia, with the veins open, in order to demonstrate that the adrenals or their nerves had not been significantly injured by this procedure. The veins were now closed by pulling gently on the ligatures while sliding the tubes against the veins, and the animal was again subjected to asphyxia or sciatic stimulation. After a period of twenty-eight to sixty seconds from the beginning of stimulation the veins were opened by releasing the pressure on them. Finally, as a check, in eleven dogs a record was taken of simple closure of the lumbo-adrenal veins or of the vena cava with no stimulation.

When these experiments were completed a post-mortem examination was made to determine whether the stellate ganglia had been completely extirpated, and whether the ligatures had been correctly placed to shut off adrenal products from the circulation.

In nineteen of the dogs operated upon the technical methods and post-mortem findings conformed to the requirements of a successful experiment. One hundred and sixty-nine observations of the result of sciatic stimulation or of asphyxia were made, which may be tabulated as follows:

I. Unopened abdomen (increases occurred in every case):

a. Sciatic stimulation; 71 observations (fig. 3).

1. Smallest increases: 6 to 12 beats per minute; 12 observations (dogs 4, 5, 17, fig. 5).

2. Usual increases: 18 to 36; 51 observations.

3. Largest increase: 84; 1 observation.

b. Asphyxia; 19 observations (16 dogs; fig. 2).

1. Smallest increase: 24 beats per minute; 2 observations.

2. Usual increases: 36 to 48; 16 observations.

3. Largest increase: 72; 1 observation.

II. Opened abdomen:

a. Sciatic stimulation; 16 observations.

1. Smallest increase: 0; 4 observations (dogs 4, 5, 17).
2. Usual increases: 12 to 20; 11 observations.
3. Largest increase: 54; 1 observation.
b. Asphyxia; 3 observations.
1. Smallest increase: 0; 1 observation (dog 17).
2. Other increases: 24 and 30; 2 observations.
c. Veins closed during stimulation.
1. Sciatic stimulation; 45 observations (fig. 4).
a. Smallest increase: 0; 35 observations.
b. Largest increases: 6 and 12; 10 observations in 5 non-fasting dogs.
2. Asphyxia; 15 observations (fig. 1).
a. Smallest increase: 0; 10 observations.
b. Largest increase: 12; 5 observations in 5 non-fasting dogs.
d. Veins opened after observations *2-c*.
1. Sciatic stimulation; 45 observations (fig. 4).
a. Smallest increase: 12; 8 observations.
b. Usual increases: 18 to 24; 34 observations.
c. Largest increase: 54; 1 observation.
2. Asphyxia; 15 observations (fig. 1).
a. Smallest increase: 12; 5 observations.
b. Usual increases: 42 to 54; 8 observations.
c. Largest increase; 66; 2 observations.
e. Closure of veins with no stimulation; 11 observations (fig. 6).
1. Vena cava (no increase in beat after opening); 5 observations.
2. Lumbo-adrenal veins (no increase in beat after opening) 6 observations.

The increase in rate during stimulation with the veins closed (II-*c-1* and *2*) occurred in the five dogs which had not undergone a preliminary fasting. Cannon (7) has cited evidence that the increase in beat under these conditons is due to a substance entering the blood from the liver. This hepatic factor is especially marked in dogs fed on a meat diet,—as were the dogs in these experiments,—but disappears after fasting.

In three of the dogs (I-*a-1*) and (II-*a-1*) only a small increase in beat, accompanied in 8 of the 12 observations by a drop in pressure (fig. 5), followed sciatic stimulation. Upon opening the abdomen, even this increase in beat was lost and sciatic stimulation, or asphyxia (1 observation), had no observable effect.

The increase in rate begins from six to eight seconds after sciatic stimulation is begun when the adrenal veins are open (*1-a* and *b*, fig. 3). If sciatic stimulation is maintained during the closure of the veins, opening the veins results in an increase in rate within four seconds (*2-d*, figs. 1 and 2). But if there is no stimulation during the closure of the vena cava or the adrenal veins, opening these veins under the conditions of our experiments did not result in an increase in beat within this time (*2-e*). An increase in beat had not occurred within twelve seconds in the record shown in figure 6. If the increases in *1-a* and *b*, and *2-d* are due to the concentration of the continued secre-

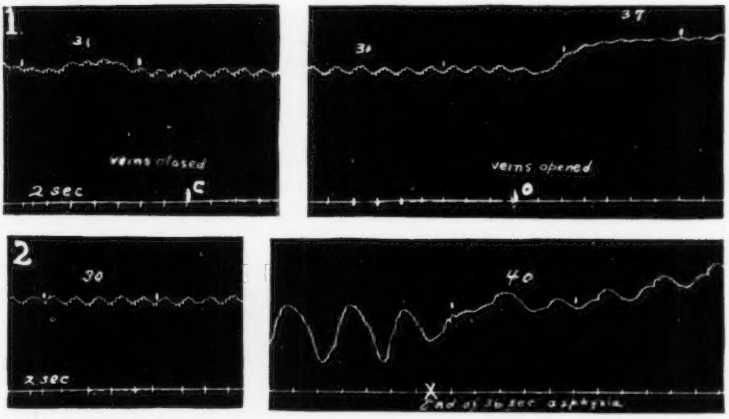

Fig. 1. Asphyxia: 40 seconds (*c* to *o*). Before asphyxia the rate is 186 beats per minute; during asphyxia, veins closed, 186; after veins are opened, 222.

Fig. 2. Asphyxia; 36 seconds (unopened abdomen). Before asphyxia the rate is 180 beats per minute; after asphyxia, 240.

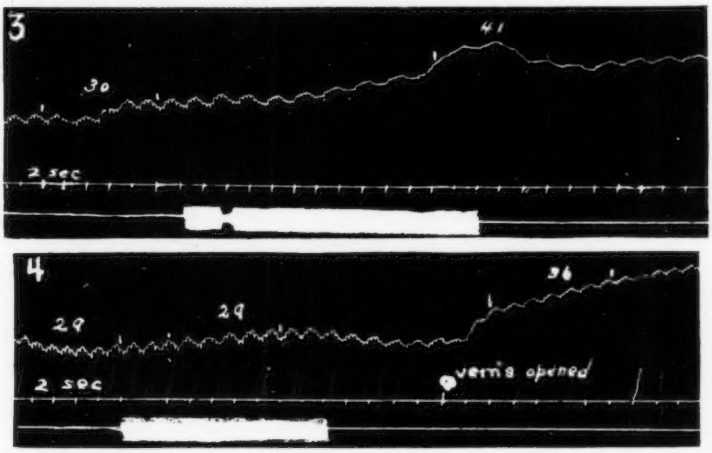

Fig. 3. Sciatic stimulation: 26 seconds (unopened abdomen). Before stimulation the rate is 180 beats per minute; after stimulation, 246.

Fig. 4. Sciatic stimulation: 18 seconds. Veins closed to *o*. Before stimulation the rate is 174; during stimulation, veins closed, 174; after veins are opened, 216.

tion of a uniform amount of adrenin, as Stewart and Rogoff have asserted (4), then there should be in *2-e* an increase in rate comparable to that occurring in *1-a* and *b*, and *2-d*. Since this increase did not occur in *2-e* it seems to the writer a safe inference that more adrenin is secreted as the result of asphyxia or sciatic stimulation. The writer concurs with the statement made by Cannon (1) that the necessary technique for demonstrating reflex adrenin discharge by the denervated heart method "is so simple that anyone inclined to doubt that more adrenin is secreted in consequence of reflex or other stimulation, may readily make the test."

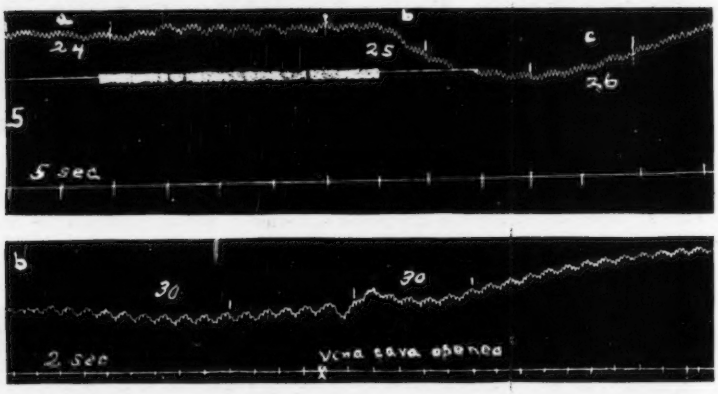

Fig. 5. Sciatic stimulation: 26 seconds. Rate before stimulation at *a* is 144 beats per minute; after stimulation at *b*, 150; at *c*, 156.

Fig. 6. Simple closure of the vena cava: 30 seconds. No stimulation. Rate closed is 180; after opening, 180.

SUMMARY

1. Sciatic stimulation or asphyxia increased the rate of the denervated heart in the dog.

2. This increase in rate was not obtained in the fasting dog when the lumbo-adrenal veins were ligated, but reappeared when these veins were again opened.

The writer wishes to express his appreciation for the stimulating criticisms of Prof. R. G. Hoskins during the course of this investigation. He is also greatly indebted to Mr. Murrell H. Turton for assistance in the operations involved.

BIBLIOGRAPHY

(1) CANNON: This Journal, 1919, l, 399.
(2) HARTMAN: Science, 1922, lvi, 146.
(3) HARTMAN, MCCORDOCK AND LODER: This Journal, 1923, lxiv, 1.
(4) STEWART AND ROGOFF: This Journal, 1920, lii, 304.
(5) GASSER AND MEEK: This Journal, 1914, xxxiv, 63.
(6) LEVY: Heart, 1913, iv, 342.
(7) CANNON AND RAPPORT: This Journal, 1921, lviii, 353.

For a more extensive bibliography the reader is referred to *Endocrinology and Metabolism*, New York, 1922, D. Appleton & Co., vol. v, 167–219.

FURTHER STUDIES OF THE REACTION OF THE SMALL INTESTINE TO SODIUM BICARBONATE

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In an earlier paper (1) we described the motor reactions of the small intestines of dogs, cats and rabbits to sodium bicarbonate when administered intravenously. The results reported were in the main confirmatory and comparable with those reported by Hammett (2) obtained from the small intestine of the albino rat, using in most of his work sodium carbonate instead of bicarbonate.

Our interest was centered mainly on the study of the types of movements set up by the alkali, but at the same time we carried out some experiments designed to locate the point of stimulation.

The results obtained indicated clearly that the locus of stimulation is peripheral in the intestinal mechanism. The central nervous system was ruled out on the basis of the facts that motor reactions were readily obtained in the animals after sectioning the vagi and splanchnics, and also in isolated segments and strips suspended in warm oxygenated saline solutions. Synaptic stimulation was not considered a possibility because the reaction was obtainable after large doses of nicotine. The results of the experiments relative to the vagus endings were somewhat indefinite. In a large number of cases the bicarbonate reaction was rather sharply antagonized by subsequent atropinization. This, however, was not a constant finding. On the other hand the administration of atropine to the point of rendering electrical vagus stimulation ineffective on the heart and intestine did not prevent our obtaining marked reactions to sodium bicarbonate. It seemed best with the data at hand to interpret these results as meaning that peripheral vagus stimulation is a factor, but that the alkali acts also on some point more peripheral. Direct stimulation of the muscle was not considered a primary factor because this tissue often responded sharply to barium after it had ceased to react at all to the sodium bicarbonate.

Enough interest and doubt attached itself to this question of the point of attack, that it seemed worth while to investigate it more fully. The object of this report is to present the results of this work.

Most of our earlier work was carried out on the intestine of the intact animal, using the balloon and tambour method for registration of movements. Our results were constant enough so that it did not seem necessary to repeat these experiments. Practically all the new data to be reported here have been obtained from the study of tissue strips and segments suspended in oxygenated fluids, plain saline, Locke's, Ringer's and Tyrode's. This technique was adopted because it seemed clear that we were dealing primarily with a peripherally acting substance. Although the environmental conditions cannot be said to be normal physiologically, yet it is probable that the factors surrounding the piece of intestine under observation can be as well, and perhaps better, controlled than when in the animal. At least modifying influences through the vagi and splanchnics, and variations in anesthesia can be excluded.

Intestinal strips and segments from dogs, cats and rabbits were used. A few frog segments were used, and also one set from a pigeon. The frog tissue reacted in a manner similar to those from the mammals, but we were not able to obtain constant reactions from those of the pigeon. We have not investigated the cause of this fact enough to enable us to account for it.

The primary and most constant reaction observed and recorded was that of increased tonus (fig. 1). This appeared in case the strip was not already sharply contracted regardless of the type of movement being executed. We have nothing new to add to the statement made in the previous paper relative to the type of movement following the initial increase in tonus. In case the tonus became very much increased, reaching a possible maximum, very little movement of any kind was recorded. In cases where the tonus reaction was less intense, movements were commonly observed, those resembling segmental types being the most common, but at times more powerful waves resembling peristaltic waves recorded from the intact animal. We were never able to control or predict the exact type of movement which would appear after the introduction of the bicarbonate. Apparently these movements are not primarily set up by the bicarbonate, but are conditioned in part, as is generally held, by tonus, which is primarily affected by sodium bicarbonate. Because of this apparent fact we have given our attention largely to the tonus reaction, attempting to determine at what point the alkali acts to bring it about.

It is well at this point to recall an earlier paragraph in which it was stated that plain saline, Locke's, Ringer's and Tyrode fluids were used as suspension fluids. All of these fluids without bicarbonate as made up are acid in reaction. The best saline we have been able to make gave a pH reading of 6.8, and usually the reading was 6.5 or 6.6. The

same holds true for Locke's, Ringer's and for Tyrode's, referring to the solutions before the addition of the bicarbonate. Muscles will relax in an environment of this reaction regardless of the care in maintaining temperature, oxygen and food supply. The addition of sodium bicarbonate will almost invariably result in an increase in tonus. That the explanation of this reaction is not wholly that the alkali neutralizes the acidity of the fluid and brings the environment of the strip up to the normal, and also that it serves to neutralize the acid formed in the muscle metabolism, is attested to by the fact that bicarbonate brings about a similar increase in tonus in the intestine of the intact animal where so far as methods available show, the environmental reaction is normal for the animal, and also by the fact that when the strips are suspended in fluid with the alkali added they react sharply on the addition of more sodium bicarbonate.

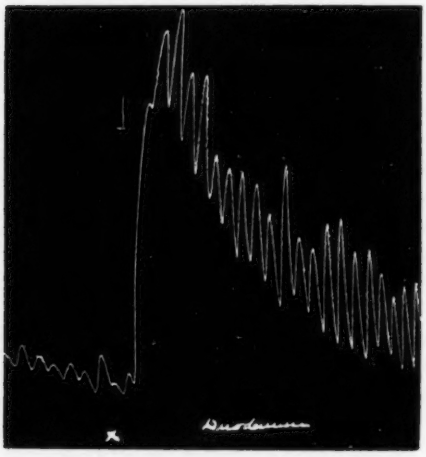

Fig. 1. Showing the most common reaction of a segment of the small intestine of the dog to $NaHCO_3$, 1 cc. of 6 per cent $NaHCO_3$ was introduced at *x*.

The only difference between the fluids above named, so far as we have been able to observe, is that rhythmical movements of the strips and segments are obtained more constantly and are maintained for a longer time in the mixed solutions than in the plain saline. This bears out the fact that rhythmical movement in smooth muscle is in part conditioned by the balance between ions, chiefly those of sodium, calcium and potassium. Relative to the tonus reaction we have not been able to note any constant and significant differences.

Does the reaction depend upon a completely intact intestinal neuromuscular mechanism? Based upon our earlier report in which it was shown that the reaction is not prevented by atropine or nicotine, the answer would appear to be in the negative. We have attempted to test out this point by separating the different muscle layers and conducting experiments with them as individual units, following in the main the technique of Magnus (3) and Gunn and Underhill (4). This gave us longitudinal muscle, circular muscle and muscularis mucosae.

These three different preparations reacted similarly when sodium bicarbonate was added to the suspension fluid. There was first a sharp increase in tonus, followed in some instances by rhythmical movements, in others by a rapid decrease to the normal tonus level without rhythmical movements (fig. 2). The appearance of rhythmical movements in the muscularis mucosae was just as constant as in either of the outer layers, but the loss of tonus was on the whole more gradual. The movements of the muscularis mucosae were recorded as described by Gunn and Underhill. The outer musculature was stripped off completely and the remaining cylinder was attached at one edge to the fixed pin at the bottom of the muscle chamber and at the upper opposite edge to the lever pin.

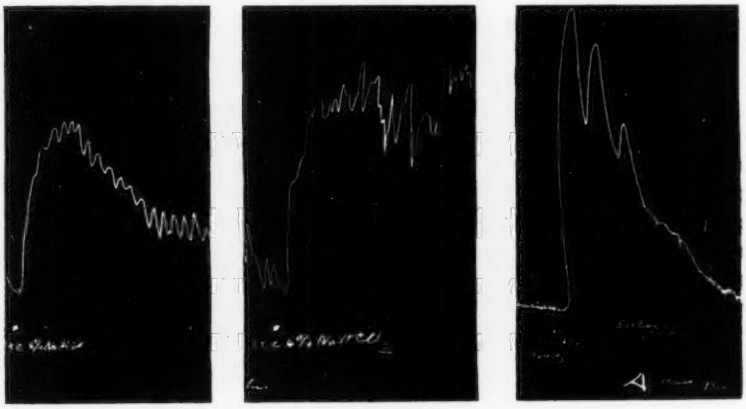

Fig. 2. Showing the effect of 1 cc. of 6 per cent $NaHCO_3$ on the separate muscular layers of the small intestine. *A*, longitudinal muscle, magnification five times; *B*, circular muscle, magnification ten times, *C*, muscularis mucosae, magnification twenty times. *C* was taken from the series on the study of the effect of cocaine on the reactivity of the muscularis mucosae to $NaHCO_3$.

These results indicate that the reaction of the intestine to bicarbonate is not primarily dependent upon a physiologically intact neuro-muscular mechanism, but that the separate units react alike.

We next proceeded to determine the effect of atropine on the reaction of the separate layers of intestinal muscle to the alkali. Just as in the case of the intact intestinal strip, the results were not uniform. In about two-thirds of the trials doses of atropine of 3 mgm. to 30 cc. of suspension fluid brought about a definite relaxation of the strips which had been contracted by sodium bicarbonate. In the remaining cases the tonus was not affected. When the procedure was reversed, and

the muscle atropinized before the addition of the bicarbonate, the subsequent addition of the alkali almost invariably resulted in a tonus reaction and in some instances an increase in rate. While it is impossible to say in any given instance what would have been the reaction without the atropine, yet based upon a large number of trials the reaction does not appear to be quite as sharp as when atropine is omitted. By using much larger doses of atropine it was possible to completely counteract or prevent the bicarbonate reaction, but when this point was reached the muscle was quite refractory to mechanical stimulation and to barium. These statements apply in particular to the longitudinal and circular muscles. In case of the muscularis mucosae the depressing effect of atropine was much less pronounced. This muscular coat also reacted to pilocarpine, and to this reaction atropine was just as strikingly antagonistic as in case of the heart or outer intestinal musculature. We have never observed or recorded any evidence that atropine stimulates any part of the local neuro-muscular mechanism of the intestine.

It seems obvious that sodium bicarbonate acts in part on points which are thrown out by atropine, but that it also stimulates at a point or points not directly affected by the atropine, probably more peripheral. The effect upon the reaction of the muscularis mucosae is significant because of the fact that vagus stimulation has not, with a few exceptions, in our series of experiments on intact animals produced any effect on the activity of the mucosal musculature. One would expect less of an effect, provided of course that we assume that the depressing effect of atropine on the intestine is primarily limited to the vagus endings.

The effect of drugs on the response of intestinal segments and strips to sodium bicarbonate. Hammett found that the intestinal segment from the albino rat responds by relaxation to sodium carbonate provided the segment has been previously treated with cocaine. He used this fact as a part of his supporting argument that sodium carbonate stimulates some peripheral point of the nervous mechanism of the intestine, and also to throw light on the explanation of his finding that segments from rats excited before killing responded by relaxation.

We tested out the reaction of intestinal segment from dogs and cats to bicarbonate after treatment with cocaine, and also tested the effect of cocaine on the alkali tonus reaction. The results were uniformly in the same direction, the degree of effect depending on the dosage. Cocainization of strips diminished or prevented the usual tonus reaction after sodium bicarbonate (figs. 2 and 3). In only three out of fifty different experiments were we able to record an initial stimulating effect of cocaine and in these instances it was very brief. When the

cocaine was introduced during the tonus reaction the segment promptly relaxed, making a record identical in appearance to that obtained on the introduction of epinephrin. The only difference, if any, was that the cocaine effect persisted for a longer time than the average epinephrin

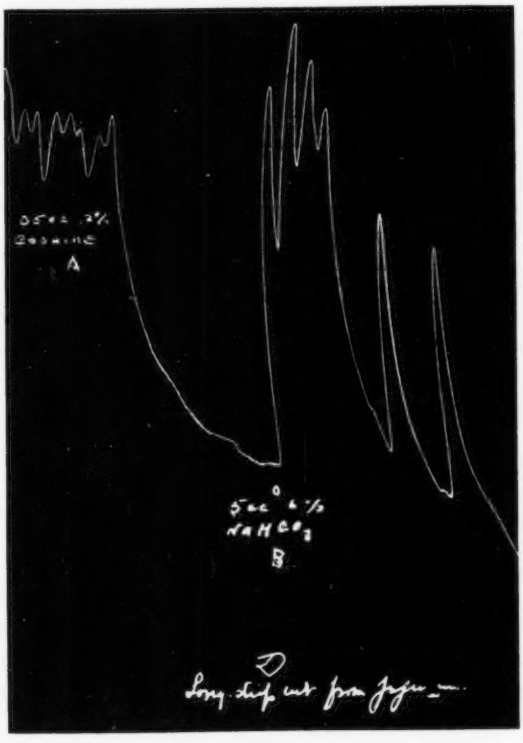

Fig. 3. The effect of a small dose of cocaine on the reaction of the intestine to sodium bicarbonate. The strip had been thrown into tonus and activity by 5 cc. of 6 per cent bicarbonate previous to the introduction of the cocaine. The second dose of bicarbonate was effective but the rate was diminished, nor was the reaction sustained as well as usual.

effect. We have only occasionally obtained a relaxation of the cocainized strips after introduction of the alkali, and in these instances the strip was relaxing at the time of the administration of the bicarbonate. Hammett used 0.1 cc. of a 1 per cent cocaine hydrochloride solution to 4 cc. of Tyrode's solution. Our muscle chamber held 30 cc. of suspension fluid. Our studies were made between a dosage range of 0.1 cc. of a 1 per cent and 1 cc. of a 20 per cent solution

added to the 30 cc. of suspension fluid. This smallest dosage produced a slight but not lasting relaxation when introduced after the bicarbonate, or when the muscle was first treated with this amount of cocaine there was little if any impairment of the reaction to subsequent bicarbonate. Doses of 1 cc. of a 1 per cent solution and over completely counteracted the effect of the bicarbonate, but left the preparation reactive to both barium and pilocarpine. With doses as large as 1 cc. of a 20 per cent solution the muscle no longer reacted to pilocarpine, barium, or to a mechanical stimulation.

A survey of the literature at our disposal relative to the effect of cocaine on the movements of the intestine shows that as a general rule small amounts of cocaine increase, and large amounts diminish the movements, and to this should be added also that tonus is affected in the same way. Papers by Anrep, (5) Bayliss and Starling (6), Langley and Magnus (7), Kuroda (8), Trendelenburg (9) and Poulsson (10) were consulted. This literature covers work on dogs, rabbits, cats and guinea pigs. According to Trendelenburg, concentrations of 1:100,000 and up depressed the intestine of the guinea pig. For the other animals on an average concentrations below 1:150,000 first stimulate then in some cases depress, while concentrations above 1:150,000 depress from the beginning. These figures are only approximate in that the limits were not always given.

The results reported by Hammett on the albino rat would indicate that the reaction of the small intestine of this animal to cocaine is similar in character to that stated for the animals above. He reported that the addition of 0.1 cc. of a 1 per cent solution of cocaine hydrochloride to 4 cc. of Tyrode's solution in successive 0.02 cc. portions brought about first a shortening of the segment with an increase in the amplitude of the rhythmical movements, followed by relaxation to the original level. The strips still kept on beating rhythmically. The point of primary interest to us in this discussion is that sodium carbonate added to the suspension fluid after cocainization brought about a relaxation of the intestinal segment instead of the usual and normal contraction. We have not been able to obtain this effect on any of the material we have used.

Does the effect of cocaine on the carbonate reaction clearly and definitely indicate the point of attack of the alkali? The answer hinges largely upon the certainty with which the cocaine action can be localized. The literature on this subject would lead one to conclude that aside from an initial slight stimulating effect it is essentially a depressant drug, affecting sensory nerves earliest, then motor, and finally muscular structures. We have not been able to consistently demonstrate an initial stimulating action on the intestine, but aside

from this our results are in accord with the general effect stated above. We were able to show consistently that the reactivity of the intestine to pilocarpine is abolished by smaller doses of cocaine than are necessary to stop or prevent a reaction to barium. The antagonistic effect of cocaine toward sodium bicarbonate also appears early and with doses smaller than necessary to appreciably lower the reactivity to barium. This would indicate that the bicarbonate acts primarily through some peripheral nervous element. Against this conclusion one might think of an early stimulation of the inhibitory mechanism on part of the cocaine.

We have employed an indirect test method relative to the question of inhibitory stimulation. The splanchnics are inhibitory to the outer musculature of the intestine, but motor to the muscularis mucosae. If cocaine stimulates during any phase of its action the inhibitory mechanism, unless it be highly selective in its action, one would expect it to bring about a motor reaction in the muscularis mucosae. It is generally known that the local application of cocaine to the intestinal mucosa causes blanching, but we have shown in earlier work that this same procedure diminished the activity and reactivity of the villi and if pushed far enough will abolish reactivity to all forms and intensities of stimulation. The effect of cocaine on suspended cylinders of muscularis mucosae is one of depression, and not of stimulation. The antagonistic action to sodium bicarbonate here is just as sharp as in case of the outer musculature (fig. 2). For this reason we are not inclined to consider stimulation of the inhibitory mechanism as accounting for the depressant action of cocaine.

How do other smooth muscle structures of the body react to bicarbonate? One series of experiments was carried out with uterine tissue, both in situ and with suspended segments. In this series were included two pregnant animals, both near term. In the pregnant animals the registrations were circular, in the others and in all the isolated segments, longitudinal.

In none of the intact animals did a dose of 50 cc. of 6 per cent sodium bicarbonate cause a definite and lasting increase in tonus or in movement. In a number of trials there was a sharp momentary shortening followed by a pronounced relaxation (fig. 4). In the other cases the relaxation followed very quickly the injection of the alkali. The effect persisted for some minutes. Succeeding injections became less effective, just as they do in case of the small intestine. This statement applies to both pregnant and non-pregnant animals. The bicarbonate brought about the same typical relaxation after ergot and after pituitrin.

Strips and segments of the dog's uterus reacted in a different manner than when intact in the animal. The addition of sodium bicarbonate

to the suspension fluid brought about an increase in tonus, and in rate and amplitude of contraction.

The effect of epinephrin was recorded in these experiments. In the four intact animals used this drug relaxed the uterus in each in-

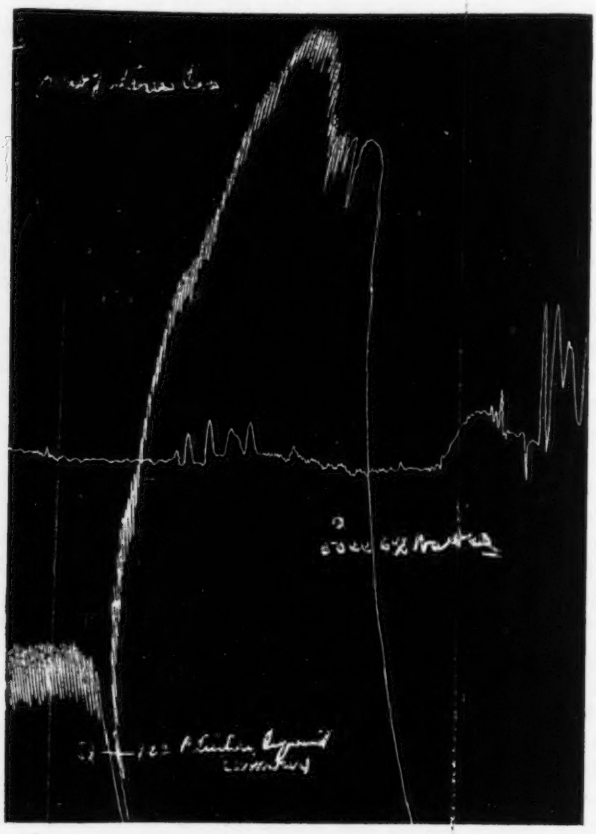

Fig. 4. The effect of sodium bicarbonate on the uterus. The uterus was thrown into activity by pituitary liquid. Tracing taken from an intact, non-pregnant dog, suspension method. Upper tracing, jejunum. Lower tracing, uterus. The intestinal writing point was set 15 mm. ahead of the uterus writing point.

stance (fig. 5). Sollmann (11) states that this drug contracts the uterus of the dog. We have observed in the pharmacological laboratory some variability in animals, so do not interpret these results as representing the reaction in every instance in the dog. The reaction in the four animals tested to sodium bicarbonate was similar to their reaction

to epinephrin. Since the reaction of the uterus to epinephrin in any animal is considered to be that obtained on stimulation of the hypogastric, our results were at first thought to point to stimulation of the hypogastric endings as the point of attack of the bicarbonate. Doubt was cast upon this conclusion when we found in our strip experiments tissues which responded to bicarbonate by contraction, and to epinephrin by relaxation.

In the face of these facts, however, it does not seem reasonable to consider sodium bicarbonate as a direct muscle stimulant. One would expect the effect to be consistently one of contraction. That the

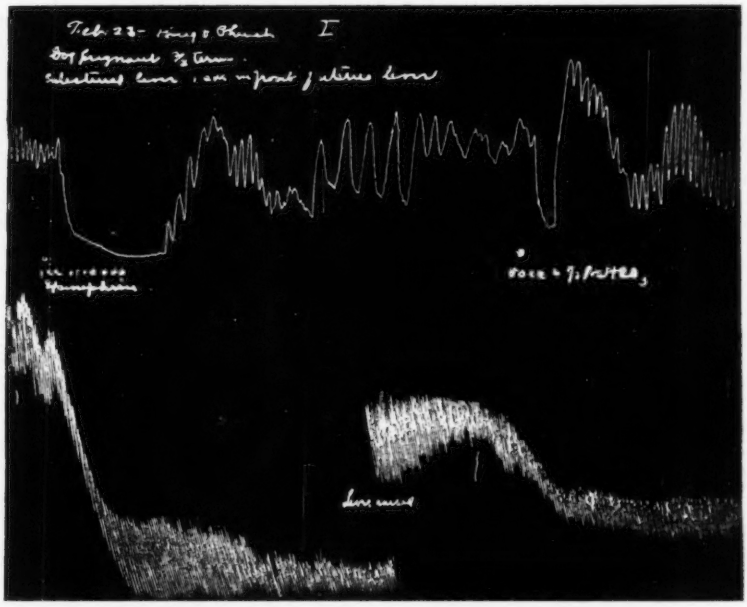

Fig. 5. Effect of epinephrin and sodium bicarbonate on the uterus and small intestine in the intact animal. Upper tracing, jejunum, lower, uterus. Respiratory movements are recorded by uterus lever.

uterus in some instances does react differently when excised than when in situ is well known, the reaction to pilocarpine being the instance to the point.

The effect of sodium bicarbonate was also tested on other muscles: the diaphragm, urinary bladder, frog's gastrocnemius, and strips from the heart of the dog.

The diaphragm responded by a sharp increase in tonus, the urinary bladder by an increase in tonus and rhythmical movement. The effect

on the gastrocnemius of the frog was very slight yet in the direction of increased tonus. We could detect no change in irritability or in its capacity for doing work.

The tests on dog's heart muscle were made on strips cut from the auricles and from the right ventricle. The animal was etherized then bled as dry as possible from the carotid arteries, finally the thorax was opened, the heart quickly excised and dropped into chilled saline. Strips were cut so as to secure maximal shortening. When suspended in warm oxygenated Locke's solution, some of the strips began beating at once, others were more tardy. The addition of 1 cc. of 6 per cent sodium bicarbonate to the suspension fluid invariably brought about an increase in tonus and rate. In several instances where the strips were tardy in starting to beat the addition of the alkali started them at once. While the effect was not so marked as when epinephrin was added, yet it was very similar in character.

A few experiments were done with spleen and with arterial rings. In each instance considerable contraction took place, but not so marked as with epinephrin.

The final series of experiments was carried out on excised segments which had been kept in the ice box at from 8°C. to 10°C. The pieces of intestine were kept in Locke's solution. After 18 hours the response of the strips was normal to sodium bicarbonate, pilocarpine, atropine, epinephrin and barium. Other portions were tested at 60 hours. The response to the above named drugs was normal in type but with the exception of epinephrin and possibly barium was diminished in intensity. The epinephrin reaction was very sharp, seemingly bearing out the findings of Gunn and Underhill that the sympathetic receptive substance in the intestine is longer lived than the parasympathetic. After 70 hours we were able to get a small tonus reaction with sodium bicarbonate, a fair pilocarpine reaction, good barium reaction, and a sharp epinephrin reaction. The sodium bicarbonate in this case brought about a small tonus reaction followed quickly by a sharp decline in tonus considerably below the normal level. Some of the 70 hour strips gave no tonus reaction with bicarbonate, but in these cases there was a slight relaxation. These strips also reacted sluggishly to barium, and in several instances to pilocarpine. After 75 hours we were not able to obtain reactions to any of the above named drugs.

These experiments were carried out on the assumption that the nerve elements in the intestine after excision would die before the muscular tissues, consequently one should be able to determine whether the bicarbonate reaction is obtainable after the segments failed to react to the autonomic drugs. The results show that the reactivity to barium persist longer than the reactivity to bicarbonate and also that

the reactivity to sodium bicarbonate and pilocarpine fails at approximately the same time.

The vitality of the inhibitory mechanism seems to be greater in the intestine than is the motor. This coupled with the observation that bicarbonate just at the reactivity borderline produces some relaxation of the intestine would suggest that it stimulates the inhibitory mechanism as well as the motor, but that normally the motor predominates.

Discussion. The conclusion that sodium bicarbonate acts primarily on the small intestine by peripheral stimulation of motor nerves is based mainly upon five facts. First, atropine in a significant number of cases brings about a diminution of tonus after the alkali. Second, cocaine is antagonistic in smaller doses than necessary to perceptibly alter the reaction to barium. Third, in all procedures the reactivity to barium persists longer than to the bicarbonate. Fourth, the uterus in intact animals both before and after the introduction of stimulating drugs reacts by relaxation. Fifth, in dying segments, the tonus reactions to pilocarpine and bicarbonate fail at approximately the same time and shortly before ceasing to show any reactivity, sodium bicarbonate brings about a relaxation instead of an increase in tonus.

We are not committed to the view, however, that the alkali does not affect the muscle directly within certain limits. A certain amount is necessary in the suspension fluid in all isolated tissue work if one wishes to obtain sustained activity. This is easily demonstrated if one compares the activity of a tissue suspended in plain saline, or in Ringer's, Tyrode's or Locke's solution without the addition of alkali, with these same fluids with the alkali included. The reaction of these fluids without the alkali is considerably more acid than the normal environment of mammalian tissue. The addition of alkali will favor better metabolism and greater permeability. We have no reason to believe that this effect is limited to either muscle or nerve alone.

That the reactions which we have described are not due entirely to the bringing up of the reaction to the physiological level is shown by the facts that the injection of sodium bicarbonate into the living animal brings about increased tonus of the intestine, and also in case the suspension fluids are first made alkaline before the tissue is introduced, the further addition of alkali brings about a sharp reaction. Hence we believe the alkali should be considered a true stimulant.

That there is another fact besides the mere improvement in metabolism in muscle is suggested by the fact that there is a wide margin of difference in response of the various musculatures of the body. The tissues from the gastro-intestinal tract are the most reactive, then come the urinary bladder and uterus, then diaphragm, then vascular tissue including the spleen, and finally skeletal muscle. All react by an

increase in tonus when excised. In case of arterial rings and spleen the reaction is almost negligible when compared with the reaction to epinephrin and barium. The reaction of skeletal muscle, both before and after curare, is in the direction of increased tone, but requires considerable magnification to record it. The action here is apparently directly on the muscle.

Assuming the action of sodium bicarbonate to be primarily on the peripheral nervous mechanism in the intestine, it seems almost useless to attempt a further differentiation owing to our limited information relative to the physiology of the plexus mechanisms in the intestine. The results with atropine would suggest that the effect is in part due to action peripheral to the vagus. This is shown best in the intact animal where it is possible to obtain sharp reactions with sodium bicarbonate after atropinization to the point where electrical vagus stimulation is no longer effective on the heart and intestine. We are mindful of the fact that it is not always easy to obtain a good reaction in the living animal by electrical stimulation of the vagi. Henderson (12) has recently published results showing that the intestinal vagus requires a much larger dose of atropine to produce paralysis than the cardiac vagus. Also it is difficult in isolated segments of the intestine to determine vagus paralysis accurately. Pilocarpine as a test drug is not entirely conclusive because in the intact animal a smaller dose of atropine is required to prevent a reaction to pilocarpine than to prevent a reaction on stimulation of the nerve electrically. We believe we can assume vagus paralysis in our series inasmuch as we used larger doses in the intact animals than stated by Henderson to be necessary, and still larger proportional doses in the work with the isolated segments.

On basis of points so far made sodium bicarbonate must act in part on some point peripheral to the vagus. This would lend itself to the view that the intrinsic nervous mechanism of the intestine is to some extent physiologically independent of the vagus, even though in origin it be vagal, which is supported by Gaskell (13) and by the more recent work of Kuntz (14).

SUMMARY

1. Segments of the small intestine of dogs, cats and rabbits, when suspended in warm oxygenated solution, manifest an increase in tonus and movement when sodium bicarbonate is added.

2. The separate layers of muscle of the intestine react to sodium bicarbonate in a similar manner.

3. Atropine is antagonistic to bicarbonate only to a limited degree. Part of the reaction to sodium bicarbonate is due to stimulation peripheral to the vagus endings.

4. Cocaine is sharply antagonistic to bicarbonate. The antagonism is complete before the reactivity to barium and pilocarpine is lost.

5. In dying segments and strips, the reactivity to bicarbonate is lost at the same time as that to pilocarpine and just before the reactivity to the barium. In the very last stages the reaction to bicarbonate is one of relaxation. This, coupled with the fact that the reactivity to epinephrin persists longer than that to pilocarpine, suggests that the bicarbonate stimulates both motor and inhibitory mechanisms.

6. The evidence presented indicates that the primary stimulating action of sodium bicarbonate is brought about by action on the peripheral part of the local nervous mechnism.

BIBLIOGRAPHY

(1) King and Church: This Journal, 1922, lxii, 459.
(2) Hammett: This Journal, 1921, lv, 414.
(3) Magnus: Pflüger's Arch., 1914, cii, 123.
(4) Gunn and Underhill: Quart. Journ. Exper. Physiol., 1914, viii, 290.
(5) Anrep: Pflüger's Arch., 1880, xxi, 38.
(6) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 99.
(7) Langley and Magnus: Journ. Physiol., 1905, xxxiii, 34.
(8) Kuroda: Journ. Pharm. Exper. Therap., 1915, vii, 423.
(9) Trendelenburg: Arch. f. Exper. Path. u. Pharm., 1917, lxxx, 98.
(10) Poulsson: Heffter, Exper. Pharm., 1920, ii, 103.
(11) Sollmann: Manual of pharmacology, 2nd ed., 1922, 325.
(12) Henderson: Journ. Pharm. Exper. Therap., 1923, xx, 99.
(13) Gaskell: The involuntary nervous system, 1920.
(14) Kuntz: Journ. Comp. Neurol., 1920, xxxii, 173.

THE MOTOR REACTION OF THE MUSCULARIS MUCOSAE TO SOME DRUGS

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During observations on villi movements, reported in an earlier paper (1), it became quite obvious that the muscularis mucosae of the small intestine of the dog manifests at times very active movements. It also seemed clear that these movements are not definitely associated with activities of the outer muscle layers.

We have never succeeded in recording these movements in the intact animal. Magnus (2) attempted to record them, using mucosal segments, but did not succeed in demonstrating definite activity. He concluded that if movements occur they must be entirely local in character. Some years later Gunn and Underhill (3), by modifying the technique of Magnus somewhat, succeeded in recording definite movements. They employed the small intestine of the cat, stripping off the outer musculature then mounting the remaining mucosal and submucosal cylinders in oxygenated Locke's solution, placing the fixed and the lever attachments diagonally. By this method they demonstrated rhythmical movements, and studied the effects of epinephrin, epinin and barium on them.

We have adopted the method of Gunn and Underhill, and have made a more extensive study of the reactions of the muscularis mucosae of the small intestine of the dog to a number of drugs.

We have employed in these studies, epinephrin, atropine, pilocarpine, pituitrin, barium, morphine, ergot, cocaine, nicotine, strychnine, nitrites and sodium bicarbonate.

The animals were handled and the segments prepared in the following manner: Ether was administered and the animal maintained under as light anesthesia as possible. When needed, segments of the small intestine were clamped off and quickly removed and prepared. A longitudinal incision was made through to the submucosa opposite the mesenteric attachment. The serosa and outer musculature were then rolled off. This requires but little force. Occasionally a few strands of circular muscle remained. These were carefully dissected off. The remaining cylinder was then mounted in warm oxygenated Locke's

solution, the fixed pin and the lever pin attached at opposite ends and diagonally to each other. Many of these preparations began to move rhythmically at once, others were more tardy.

For some reason which we do not definitely know, the muscularis mucosae seems shorter-lived and more sensitive to rough handling, particularly stretching, than the outer musculature. For this reason we selected only preparations which had been subjected to a minimum of trauma.

A lever magnification of twenty times was used. The muscle chamber held 30 cc. and, unless otherwise stated, the experiments on which the following findings are based were carried out with this volume of Locke's solution.

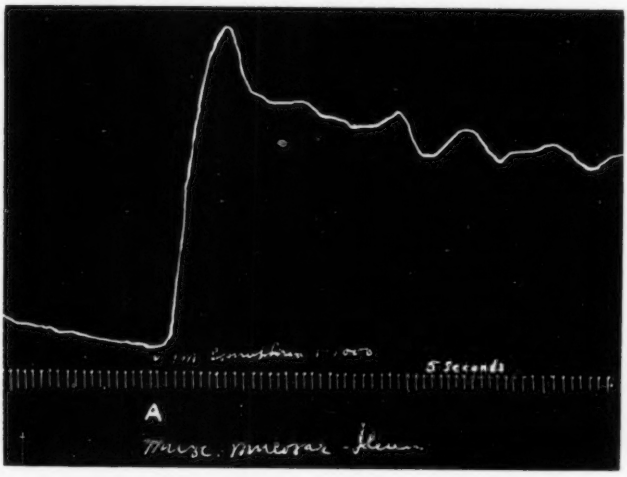

Fig. 1. Muscularis mucosae of ileum. At *A* 1 minim of 1:1000 epinephrin was introduced into the suspension fluid.

Epinephrin. The introduction of epinephrin into the environment of the mucosal cylinder invariably caused it to contract. This tonus reaction was proportional to the amount of drug used up to the point of maximal shortening. It was also as a rule followed by rhythmical movement, unless the tonus was too great, in which case the rhythmical movement did not appear until some relaxation had taken place. In preparations which manifested rhythmical movements before the application of the drug, the amplitude of the contractions was increased by the drug, but the rate was not affected. In a number of tests where there were no movements to begin with, the introduction of epinephrin started them (fig. 1).

These results are in harmony with the findings in the intact animal. Stimulation of the splanchnics causes a retraction of the villi and a ridging and grooving of the mucosa as a whole. The effect of epinephrin is presumably due to stimulation of these autonomic endings.

Atropine. In the intact animal the injection of atropine produces no sharp effect on the movements of the mucosa so far as can be detected

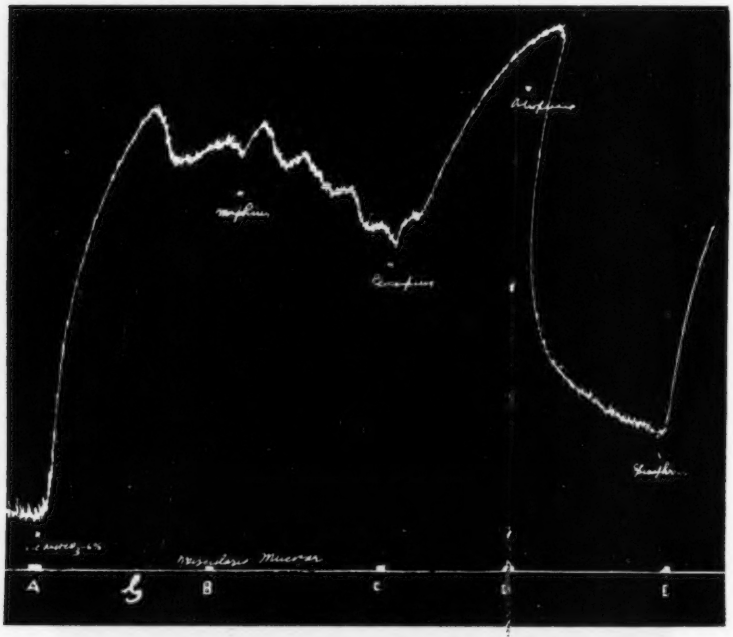

Fig. 2. Record from muscularis mucosae of jejunum. *A*, 1 cc. 6 per cent $NaHCO_3$. *B*, 15 mgm. morphine sulphate. *C*, 3 mgm. pilocarpine hydrochloride. *D*, 1 mgm. atropine sulphate. *E*, 2 minims 1:1000 epinephrin. The small rapid movements of the writing point are due to aeration vibration.

by direct inspection. There is, however, a gradual increase in villi movements, requiring some minutes to reach the maximum, then gradually diminishing to the original state of activity.

With mucosal cylinder preparations we were not able to obtain results which corresponded fully to the above. If the preparations were relaxed at the time of the introduction of the atropine no change was recorded. On the other hand, with preparations showing considerable tonus the introduction of the drug was followed most commonly by relaxation. This practically always occurred if the contraction had been brought

about by pilocarpine, but not so constantly after the use of epinephrin and sodium bicarbonate. We never recorded any stimulating effect of atropine which could account for the gradual increase in villi movement observed in the live animal as mentioned above (fig. 2).

Pilocarpine. The response of the muscularis mucosae to pilocarpine was invariably by an increase in tonus, and most commonly by an increase in the amplitude of movement. The rate was not regularly affected but where this did occur it was in the direction of an increase. The most effective dose was 3 mgm. Doses larger than this left the preparation in a state of inactivity and refractory to all types of stimulation. The action of pilocarpine was sharply antagonized by atropine (fig. 2).

The results with pilocarpine, in the light of the results reported in an earlier paper, present several peculiarities. Stimulation of the vagi in the intact animal does not affect the motor activity of the intestinal mucosa in the dog. This would put the muscularis mucosae in a class with the sweat mechanism and the uterus insofar as the response to pilocarpine is concerned. We also noted in the earlier work cited that pilocarpine aroused villi activity after atropinization. The sharp antagonism between atropine and pilocarpine which we have shown with the mucosal cylinders would suggest that the stimulating effect of pilocarpine on villi activity in the live animal is due not to a direct action on the local motor mechanism, but to some secondary factor, possibly to some change in blood or lymph flow.

Nicotine. In making observations on villi movements, referred to earlier, it was found that the injection of nicotine into the circulation of the dog causes a sharp retraction of the villi, and a marked grooving and ridging of the mucosa as a whole. Nicotine was classed as a powerful motor stimulant of the intestinal mucosa. The results of the tests with nicotine on mucosal cylinders are somewhat confusing. A considerable number of preparations did not respond at all, others responded by giving a small tonus reaction which was of brief duration. In the live animal the effect of nicotine was of the same character and apparently just as sharp as the reaction to epinephrin. With the isolated preparations the results were not at all comparable. Repeated doses of nicotine made the preparation refractory to other forms of stimulation, earlier to epinephrin, later to barium. It is worthy of note at this point that previous treatment with strychnine made the cylinders more reactive to nicotine.

The generally accepted view is that nicotine first stimulates, then depresses the autonomic ganglia. The results just cited suggest that the effects of nicotine in the live animal are in part due to stimulation of synapses in the superior mesenteric ganglia and in the local plexuses

of the intestine. The former does not entirely account for it because the mucosa in the live animal reacts sharply after preganglionic stimulation of the splanchnics ceases to produce any effect. In the isolated cylinders the only ganglion cells present are those of Meissner's plexus. This would lead one to conclude that the functional activity of Meissner's plexus is in part determined by impulses from Auerbach's plexus.

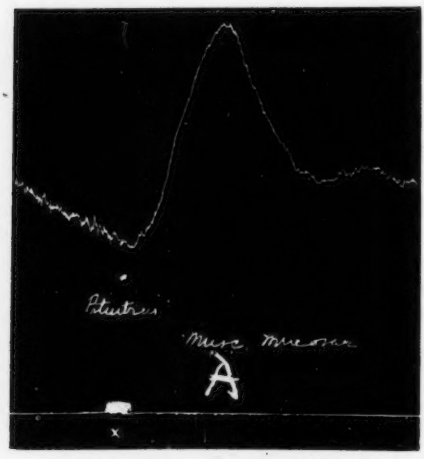

Fig. 3. Muscularis mucosae of jejunum. At *X* 5 minims of pituitrin (O) were introduced into the muscle chamber.

Pituitrin. The effects of this drug also proved different when applied to the cylinder preparations than when injected into the living animal. Pituitrin caused isolated cylinders to shorten (fig. 3). In the living animal we never observed any increase in villi movement, any retraction, or any definite change in the mucosa as a whole except a blanching effect of short duration. We have no satisfactory explanation for this difference in effect.

Barium. The effect of barium was consistently one of stimulation. The reaction consisted in both an increase in tonus and in rate. The amplitude was in most cases diminished, this depending somewhat on the tonus. Large doses of barium killed the preparations.

Morphine. Morphine was without effect either on tonus or rhythm, except in very large doses in which cases it acted as a depressant. Doses up to 15 mgm. were without effect. Doses larger were depressant, although in several instances 40 mgm. did not prevent a reaction to barium (fig. 2).

Ergot. Ergot consistently caused moderate stimulation, affecting mainly the tonus. The fluid extract was used in doses from 8 minims to 1 cc. We do not consider the alcohol in this range of dosage to be a factor of importance, because the introduction of corresponding amounts of alcohol did not affect the activity of any of the cylinders tested.

Cocaine. The effect of cocaine on the pupil is explained usually on the basis of peripheral sympathetic stimulation (4). The same is true for the blanching effect when the drug is applied to mucous membranes.

Since the splanchnics supply motor fibers to the intestinal muscularis mucosae, one would expect to be able to demonstrate some stimulating effect of cocaine by using the proper dosage.

Concentrations from 1:300,000 to 1:1,500 were used. In two instances out of thirty-two trials were we able to record a definite primary stimulating effect of cocaine, and in both instances the dosage was approximately 1:300,000.

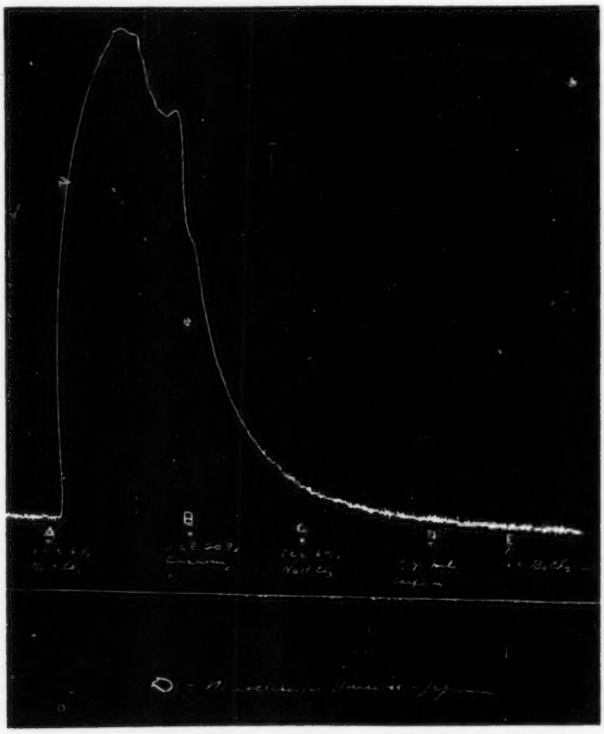

Fig. 4. Record from the muscularis mucosae of the jejunum, showing the depressant effect of a dose of 1:1500 cocaine hydrochloride.

The outstanding effect of cocaine was that of depression. This drug acted as a sharp antagonist to all drugs studied which acted as stimulants, including barium. In the case of barium, however, the antagonistic dose was much larger than was necessary, for instance, against epinephrin or sodium bicarbonate (fig. 4).

Strychnine. The effects of strychnine were variable. Doses from 0.5 mgm. to 5 mgm. were tried. When effects were recorded they were

in the direction of increased tonicity, in several instances this was quite marked. In more than half of the trials no change of any kind was recorded. Note has already been made of the effect of previous strychninization on the subsequent nicotine reaction (fig. 5).

Nitrites. Two series of tests were run, one with 10 mgm. doses of sodium nitrite, and the other with 1 mgm. doses of nitroglycerine. No essential differences were noted in the effects, both being depressant and antagonistic to all stimulants (fig. 6).

Sodium bicarbonate. The effect of sodium bicarbonate

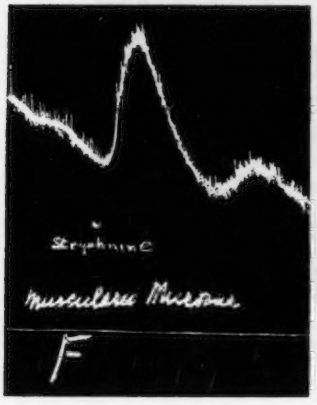

Fig. 5. Effect of 1 mgm. of strychnine sulphate on the muscularis mucosae of the jejunum.

Fig. 6. Muscularis mucosae of jejunum. At *A* 1 cc. of 6 per cent $NaHCO_3$ was introduced, at B $\frac{1}{100}$ gr. nitroglycerine. The muscle recovered rapidly from the effects of the nitroglycerine.

was one of stimulation, bringing about both an increase in tonus and in rate of movement. The most effective dose was found to be 1 cc. of a 6 per cent solution added to 30 cc. of Locke's solution. The effect up to a maximum was clearly proportional to the amount used. Much larger doses than 1 cc. brought about a sharp tonus reaction without much rhythmical movement, followed by a rapid decline in tonus. These cylinders were, after this treatment, rather refractory to subsequent additions of bicarbonate, even after washing, but reacted well to barium (fig. 7).

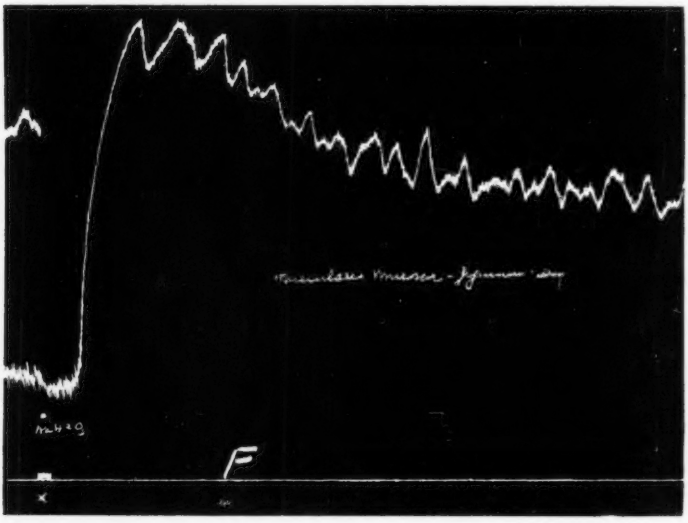

Fig. 7. Muscularis mucosae of the jejunum. At *X* 1 cc. of 6 per cent $NaHCO_3$ was introduced.

SUMMARY AND CONCLUSIONS

1. Evidence is presented showing that the muscularis mucosae of the dog's small intestine executes rhythmical movements when suspended in a proper manner in warm aerated Locke's solution.

2. Epinephrin, pilocarpine, sodium bicarbonate and barium are vigorous stimulants of the muscularis mucosae.

3. Ergot, nicotine, pituitrin and strychnine are mild stimulants. Of these strychnine is the most inconstant. The stimulating effect of nicotine is increased after strychnine.

4. Morphine, nitrites and cocaine are depressant. Very small doses of cocaine may cause some initial stimulation.

5. Atropine is depressant after stimulating drugs, the effect being most marked after pilocarpine, and less so after epinephrin and sodium bicarbonate.

BIBLIOGRAPHY

(1) KING AND ARNOLD: This Journal, 1922, lix, 97.
(2) MAGNUS: Pflüger's Arch., 1904, cii, 356.
(3) GUNN AND UNDERHILL: Quart. Journ. Exper. Physiol., 1914, viii, 290.
(4) SOLLMANN: Manual of pharmacology, 1922, 301.

THE EFFECT OF INSULIN ON THE CENTRAL NERVOUS SYSTEM AND ITS RELATION TO THE PITUITARY BODY

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The typical convulsions shown by rabbits whose blood sugar has been lowered to about 0.045 per cent by the injection of insulin, as described by previous investigators working on the effects of this pancreatic extract (1), appear to be somewhat similar to those produced in strychnine poisoning. The effect of this drug on reciprocal innervation is well known through the experiments of Sherrington. Instead of the relaxation of one antagonistic muscle simultaneous with the contraction of the other, both muscles contract. This experiment is most easily performed on decerebrate cats. In attempting to discover whether this condition held in the insulin convulsion, we encountered the difficulty of being unable to cause a lowering of the blood sugar in the decerebrate cats to the level which had been found necessary to produce convulsions in rabbits. This is not due to generic differences between cats and rabbits since typical convulsions can be induced in normal cats by subcutaneous injection of insulin, the dose being about 0.3 cc. per kilogram of body weight. The following is the description of one case.

Cat 6. Weight 2.6 kgm.

12:30 p.m. Eight tenths cubic centimeter insulin injected subcutaneously.

3:00 p.m. The cat is becoming weak and comatose; lies on its side and is hard to rouse; emits a peculiar cry; has tremors in hind limbs; pupils of eyes dilate and constrict in rapid succession; defecates and urinates.

3:10 p.m. Cat has become hypersensitive; twitching of both front and hind limbs; responds to auditory stimuli such as whistling by opening its eyes wide, pupils becoming greatly dilated; knee jerks elicitable on the slightest touch; gives flexion reflex when toes are pinched; respirations rapid and shallow; salivating freely; defecates.
Appears uneasy, raising its head. Blood sugar at this time was 0.046 per cent.

3:20 p.m. Respirations very rapid and shallow; muscular twitching all over the body; head is thrown back; pupils widely dilated; front legs rigid and extended, hind legs limp; makes walking movements with front legs; rolled over to right once or twice; cries continuously, salivating freely; whirling does not produce nystagmus. Blood sugar 0.043 per cent.

3:25 p.m. Respirations extremely rapid and shallow; cries; head thrown far back; front legs rigid, hind legs limp, tail shows some rigidity; makes walking movements with front limbs; rolls over and over to the right emitting this peculiar cry all the time; the hair, particularly that along the back, stands up straight. Blood sugar 0.036 per cent.

3:30 p.m. Ten cubic centimeters saturated dextrose solution injected subcutaneously.

3:55 p.m. Front limbs appear normal; hind limbs are weak and show some incoördination; can sit up, but sways slightly from side to side.

3:58 p.m. Six cubic centimeters saturated dextrose solution injected subcutaneously. Animal fairly normal though rather weak.

9:00 p.m. The animal was found in convulsions again. Ten cubic centimeters of saturated dextrose solution and 1 cc. epinephrin injected subcutaneously.

9:00 a.m. The animal has apparently entirely recovered except that it walks slower than normally.

It was suggested by Dr. J. J. R. Macleod that the high level of the blood sugar in decerebrate cats might be due to the action of the pituitary body. In order to test this, a series of cats was decrebrated in the usual manner, the cut passing slightly in front of the anterior corpora quadrigemina, not only to leave the pituitary body intact but also to avoid injury to the eighth nerve. The latter was shown to be in good condition by the response of the cat to auditory stimuli such as whistling, clapping of the hands, etc. In some cats the pituitary body was left untouched, and in others it was wholly removed by careful dissection. The condition was verified later at autopsy.

It was found that if the insulin was injected immediately after the removal of the pituitary body, an insulin convulsion ensued. The convulsion was characteristic, showing the rolling over, swallowing movements, defecation and urination. There was no phonation or salivation as these are not possible in decerebrate cats, also the hair did not rise.

In estimating the blood sugar, blood was taken from the carotid artery and the percentage calculated by the Shaffer-Hartmann method. It was noted that in all cases where the blood sugar was low, the blood was very dark and venous in character—in fact, the approach of the blood sugar to the convulsive level could be foretold by the color and character of the blood. In all our experiments except cat 2, the animals were starved 18 to 48 hours before the operation of decerebration or decapitation.

From table 1 it will be seen that when the pituitary body is left intact the blood sugar maintains a constant high level or may actually rise. Under these conditions, although the injection of insulin causes a fall in the blood sugar, in the most extreme case (cat 5) it was still

far above the convulsion level. Cat 2 was not starved before the experiment and it will be noted that insulin causes very little effect, though the animal was unable to survive a second injection. It is much easier to bring the blood sugar down to the convulsive level in a starved than in a well-fed animal, because there is no store of glycogen in the liver which can be utilized to restore glucose to the blood. (Cf. effect in rabbits (2).)

TABLE 1

Decerebrate cats—pituitary body intact

CAT NO.	BLOOD SUGAR PERCENTAGE AT HOUR INTERVALS FOLLOWING DECEREBRATION				
	1	2	3	4	5
20♂	0.320	0.390	0.366	0.350	
30♂		0.316	0.298	0.320	0.320
42♂		0.258	0.260	0.400	0.360
5♀	0.230	0.270	0.300*	0.170	0.160
24♀	0.248	0.310	0.350	0.370*	0.285
2♂	0.240*	0.240	0.235	0.200*	

* Insulin injected at the time indicated.

TABLE 2

Decerebrate cats—pituitary body removed

CAT NO.	BLOOD SUGAR PERCENTAGE AT HOUR INTERVALS AFTER DECEREBRATION											
	1	2	3	4	5	6	7	8	9	10	11	12
44♀		0.250	0.172	0.120	0.101	0.084	0.116	0.117	0.112	0.111	0.114	0.117
6♀	0.230	0.212*	0.124	0.080	0.058*	0.052						
28♂	0.170*	0.136	0.084	0.072								
29♀	*		0.048†	0.026†	0.040†							
32♀	*		0.040†	0.020†	0.030†							
41♀			0.250	0.310	0.248	0.222	0.270					

* Insulin injected.

† Convulsions occurred at this point.

Table 2 shows that in decerebrate cats with the pituitary body removed, although the blood sugar immediately after decerebration is comparatively high, it does not become higher or even maintain its high level. In cat 44 the normal was reached after three hours, and was maintained fairly constant, after an initial drop, for eight hours. This initial rise is probably due to the ether anesthesia, as it is known that the percentage of blood sugar rises during ether narcosis (3). The blood sugar in the decerebrate cats which showed convulsions (cats 29 and 32) reached the low level of 0.026 and 0.020. The effect

seemed most readily produced by injecting insulin immediately after decerebration. In this table we have included cat 41 which was intended to serve as a control animal like cat 44. The maintenance of the high blood sugar is due to the fact that the animal was partially asphyxiated by constant gathering of mucus in the tracheal cannula throughout the entire experiment. It showed great difficulty in breathing and frequently had slight convulsions. This is the usual effect of asphyxiation (4), for the blood sugar in a rabbit will rise in five minutes from normal, i.e., about 0.120 per cent, to 0.225 per cent by clamping the trachea until an asphyxial convulsion takes place.

Since epinephrin acts in an opposing manner to insulin (5) it was thought that the adrenals and the pituitary body might act in conjunction with one another to counteract the effect of insulin. Consequently, in two decerebrate cats the pituitary body was removed and in addition the adrenals were tied off by means of mass ligatures.

TABLE 3

Pituitary body removed and adrenals tied off

CAT NO.	BLOOD SUGAR PERCENTAGE—TIME IN HOURS					
	1	2	3	4	5	6
23♂	0.230	0.176	0.150*	0.090*		
27♀	0.184	0.148	0.118	0.110	0.090	0.097

* Insulin injected.

Table 3 shows that the adrenals evidently play little part in maintaining the high blood sugar level. This confirms the observations of Stewart and Rogoff (6).

The typical convulsion both in the normal and in the decerebrate cats seems to be always associated with disturbances in the medulla of the brain. This is evidenced by the salivation, phonation, rapid respiration, loss of control of the sphincters, etc. The respiratory center seems to be affected most of all since those animals to which an overdose of insulin has been given die of respiratory failure, and their arterial blood becomes very dark and venous in character. Since the convulsion is the same in decerebrate as in normal cats (except for phonation and salivation) the effect of the insulin cannot be on the cerebral hemispheres, for in a decerebrate cat these are entirely removed. In order to determine whether the spinal cord was affected by insulin a series of cats was completely decapitated and kept in proper experimental condition for hours by means of artificial respiration.

Table 4 shows that the blood sugar of decapitate cats has the same initial high level as the decerebrate cats and that ther is a gradual

falling off toward the normal as in decerebrate cats with the pituitary body removed. The initial high level is probably due to the action of the ether anesthesia.

Cats 31 and 33 formed an interesting comparison. They were identical in very way, having been obtained from the same litter and both were in perfect condition when the experiment started. These cats were subjected to exactly the same conditions as to aeration, etc., but cat 33 received insulin whereas cat 31 did not. The blood from cat 31 appeared over-aerated, was very bright red in color and did not clot easily. On the other hand, in cat 33 the samples of blood taken in the neighborhood of the convulsive level appeared under-aerated, were very dark in color and clotted easily. The latter is characteristic of the blood of both normal and decerebrate cats taken during the period of convulsions.

TABLE 4

Decapitate cats

CAT NO.	BLOOD SUGAR PERCENTAGE—TIME IN HOURS											
	1	2	3	4	5	6	7	8	9	10	11	12
31♂	0.260	0.298	0.284	0.172	0.132	0.113	0.090	0.070	0.070	0.090		
19♂		0.220	0.088	0.100	0.120*	0.104	0.062					
21♀	0.188	0.190	0.180	0.168*	0.100	0.086	0.082					
33♂	*		0.046	0.036	0.051							
35♀	*		0 028	0.040	0.070	0.068	0.016	0.028	0.060	0.083	0.080	0.091

* Insulin injected.

Although the blood sugar was maintained below the convulsion level in the decapitate cats for long periods of time, particularly in cat 35, there were no signs whatever of convulsion and the animal continued to act like an ordinary decerebrate preparation for over twelve hours without having to inject dextrose. A normal or decerebrate cat could not have lasted so long with a low percentage of blood sugar. Since there was no apparent difference in behavior of the decapitate preparation after injection of insulin it is probable that insulin has no direct effect on the nerve cells of the spinal cord. This is quite different from the action of strychnine, for when this drug is injected into a decapitate cat, the animal becomes hypersensitive, almost leaping off the table at a touch, so exaggerated are the reflexes, and the reciprocal innervation is lost so that both antagonistic muscles contract simultaneously.

The fact that leaving the pituitary body intact caused the blood sugar to remain at a high level, raised the question whether the

extract from this gland when injected into pituitary-less decerebrate cats would have the same effect. The pituitary body has been shown to exercise an effect on sugar metabolism (7) and it has been reported that subcutaneous as well as intravenous injections of pituitary extract into normal animals causes a rise in blood sugar (8).

Our first series of experiments in which pituitrin was injected subcutaneously gave negative results. There appeared to be no effect on the blood sugar no matter what amount of pituitrin was injected. The failure of the pituitrin to cause change in blood sugar was not due to the use of an inactive preparation of the extract since in each case the effect on the movements of a piece of rabbit intestine was unmistakable. The lack of response proved to be due to the method of administration. On injecting the pituitrin intravenously, we obtained a marked effect. In these experiments the first sample of blood was not taken until three hours after decerebration. Pituitrin was

TABLE 5

CAT NO.	BLOOD SUGAR PERCENTAGE—TIME IN MINUTES AFTER DECEREBRATION															
	180	190	200	210	220	230	240	250	260	270	280	290	300	310	400	445
S♀	0.248	a			0.268		+	0.380	0.348	0.368		+	0.400			
38♀	0.392		+	0.362	0.400	0.348			0.300	+	0.368	0.320	0.310		0.336*	0.276
7♀	*0.056	+	0.093	0.060	0.053		0.040	+	0.086	0.056	0.050					
39♂	*0.046				0.024	+	0.060	0.046	0.024	c	+	0.053	0.028	0.020		

a Asphyxial convulsion due to mucus in tracheal cannula.
+ Pituitrin 0.5 cc. injected intravenously.
* Insulin injected immediately after decerebration.
c Insulin convulsion.

then injected and three samples of blood were taken at ten-minute intervals. The experiment was repeated after a wait of half an hour.

From table 5 it will be seen that pituitrin injected intravenously causes an immediate rise in the blood sugar which, however, is not maintained, for in each case there was a falling off within half an hour. The effect was well shown in cats 7 and 39. Before each injection of pituitrin these animals were exhibiting all the preliminary signs of an oncoming convulsion, such as increase in rate of respiration, restlessness, muscular twitchings. Immediately after the injection of pituitrin these signs all disappeared, the respirations became deeper and slower, the cats became quieter and the twitchings disappeared. This effect may be due to the raising of the blood sugar alone or due to a direct antagonistic action of the pituitrin against the insulin. Burn (9) believes the latter to be the case.

The cat 38 was a pregnant female, the fetuses being at nearly full term. It will be noted that although the pituitary body was removed

the blood sugar percentage three hours after decerebration was of the same order as in the decerebrate cats with the pituitary intact. This may be a similar phenomenon to that observed by Carlson and Ginsburg (10) on depancreated pregnant dogs.

CONCLUSIONS. From these experiments it appears that the bulbar centers are affected by injection of insulin since typical insulin convulsions can be induced in decerebrate cats whose cerebral hemispheres have been entirely removed, but not in decapitate cats, although in the latter case the blood sugar may be reduced below the convulsion level and maintained there for a considerable time. The pituitary body, if left intact in the decerebrate cats, causes a high level of blood to be maintained, so that normal doses of insulin are unable to lower the blood sugar sufficiently to cause a convulsion. Intravenous injection of commercial pituitrin also antagonizes the action of insulin and is able to cause the symptoms of an oncoming convulsion to disappear temporarily.

As to the precise way in which insulin produces its effect on the bulbar centers and gives rise to the characteristic symptoms of the insulin convulsion, our experiments suggest that anoxemia is an important factor. There are many points of resemblance between an insulin convulsion and an asphyxial convulsion such as that caused by clamping the trachea of a cat or rabbit. In both cases the animal first runs about blindly, bumping into any obstruction; it then falls on its side making vigorous running movements with the fore legs, the hind legs being often tightly flexed against the abdomen; and in some cases the rolling over has been observed in an asphyxial convulsion. Rabbits as well as cats show similarity in the two types of convulsion. This behavior as well as the fact that the arterial blood from an animal in an insulin convulsion is very dark and venous in character may indicate that the action of insulin on the medulla is a secondary effect rather than a direct one upon the nerve cells of this region. It may possibly be that through the lowering of the blood sugar certain oxidative processes become depressed to such a degree that the brain cells, which are known to be especially susceptible to lack of oxygen, are affected in much the same manner as in asphyxia. It is as yet impossible to conjecture whether chemical changes in the blood or functional changes in the pulmonary cells of the lungs might be responsible. Investigation is now going on in this laboratory in the endeavor to throw light upon this point.

SUMMARY

1. Decerebrate cats with the pituitary body left intact maintain a high blood sugar level which is not materially reduced by insulin.

2. Typical insulin convulsions can be induced in decerebrate cats whose pituitary body has been removed.

3. The presence or absence of the adrenal bodies does not appear to have marked influence on these results.

4. The blood sugar percentage in decapitate cats may be lowered and maintained below the convulsion level of normal cats, yet no signs of convulsions be observed.

5. Commercial pituitrin injected intravenously into pituitary-less decerebrate cats appears to have a similar effect as leaving the pituitary body intact, though to a less degree.

6. The locus of action of insulin is on the bulbar centers, particularly the respiratory center.

BIBLIOGRAPHY

(1) Banting, Best et al: Journ. Metabolic Res., 1922, ii, 125.
(2) McCormick, Macleod, Noble, O'Brien: Journ. Physiol., 1923, lvii, 234.
(3) Ross and Davis: This Journal, 1921, liv, 474.
(4) Yamakami: This Journal, 1919, l, 177.
(5) Banting, Best, Collip, Macleod and Noble: This Journal, 1922, lxii, 559.
(6) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1920, xv, 238.
(7) Cushing: The pituitary body and its disorders, 1912, 17.
(8) Parton and Katz-Klein: Zeitschr. f. d. gesammt. exper. Med., 1921, xxv, 98.
(9) Burn: Journ. Physiol., 1923, lvii, xxxviii.
(10) Carlson and Ginsburg: This Journal, 1915, xxxvi, 217.